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Virology

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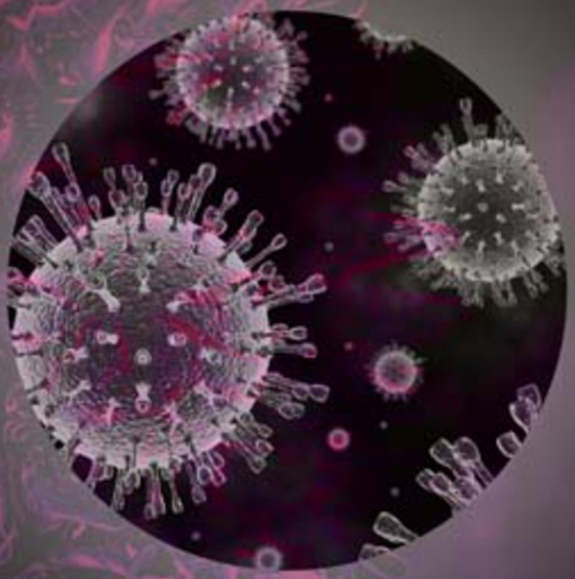

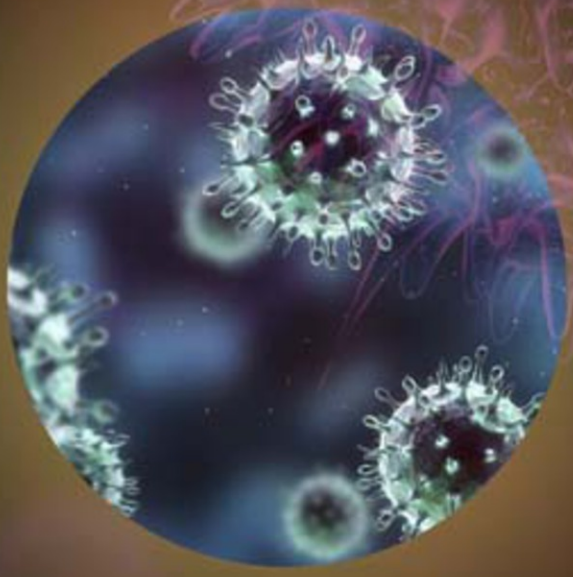

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Virology

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This book is dedicated to Professors Richard S. Tedder, Hans Wilhelm Doerr and Robert Swanepoel, without whom the world of viruses would be far less exciting.

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Virology

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Preface

Clinical virology can be a mysterious subject, the realm of organisms too small to see, intangibles that affect us and those around us every day. From the common cold to vomiting and diarrhoea outbreaks in hospitals and on cruise ships, to cancer, viruses affect our lives in numerous ways.

Grasping that which we are unable to see is, to many, like trying to grasp the unknowable. Perhaps this explains why, historically, viruses have been deemed to be insignificant, and relegated to the unimportant. However, over the past decade a number of factors have increased interest in virology as a medical discipline. Virology has been at the forefront of the medical application of molecular technology, viral infections have been an important cause of mortality and morbidity in patients treated with new immunosuppressive therapies, spurring interest in diagnosis and the development of antiviral agents; whilst rapidly developing epidemics like SARS and avian influenza, and more slowly developing but devastating epidemics like HIV, have dominated much of the medical and popular literature over the past few years.

The purpose of this book is to provide an engaging and concise introduction to medical virology for all who are interested to know more – from student scientists and health professionals to specialists from other fields wanting to broaden their knowledge on this subject. Systematic and clinical topics give the reader insight from both a basic viral and a clinical/syndromic perspective. Concise descriptions with a predominance of clear and simple illustrations allow this subject to be accessible to all, whilst also providing a solid foundation for those who are planning to delve deeper into this fascinating field.

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Viruses

Viruses – Characteristics and structure

The origin of viruses

There have been three major theories about the origins of viruses. The **regressive**, or reduction, hypothesis suggests that viruses began as small cells that, much like bacteria such as *Chlamydia*, infect larger cells. These pre-virus cells then lost their metabolic and most of their reproductive abilities, and became inert outside of a cellular environment, and reliant on cellular pathways for reproduction. The **escape**, or cellular, hypothesis suggests that mobile elements such as retrotransposons obtained genes encoding capsid proteins and enzymes, and, much like plasmids we know today, were able to escape from their original cellular environment and move to, and replicate in, other cells. The last hypothesis is the **co-evolution**, or virus-first, hypothesis, which states that both cells and viruses evolved alongside each other.

The regressive and escape hypotheses predict some similarity between genes for viral structural proteins and cellular genes, yet there is no such similarity except for cellular genes that have been incorporated into existing viruses. However, all viral replication genes are distantly related, while all modern cells share related genes. The co-evolution hypothesis predicts that viruses for the three domains of life – **Archaea**, **Bacteria**, and **Eucaryota** – would have some genetic similarity to their hosts, and differ from viruses infecting other

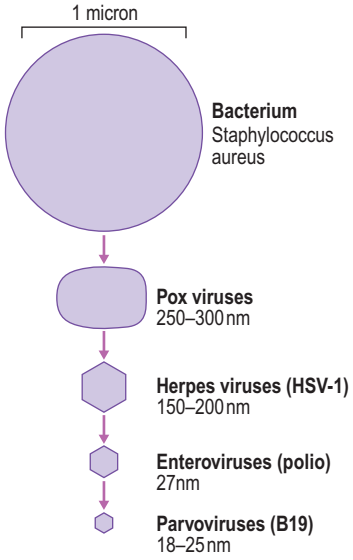


Fig. 1 **Sizing up.**

Table 1 **Viruses vs. bacteria**

	Bacteria	Viruses
Size	Larger	Smaller (20–400 nm)
Visualised with light microscope	Yes	No, except for poxviruses /mimiviruses
Growth on artificial media	Yes	No
Contain both DNA **and** RNA	Yes	No
Division by binary fission	Yes	No
Ribosomes present	Yes	No
Contain muramic acid	Yes	No
Sensitive to antibiotics	Yes	No
Motility	Yes/No	No

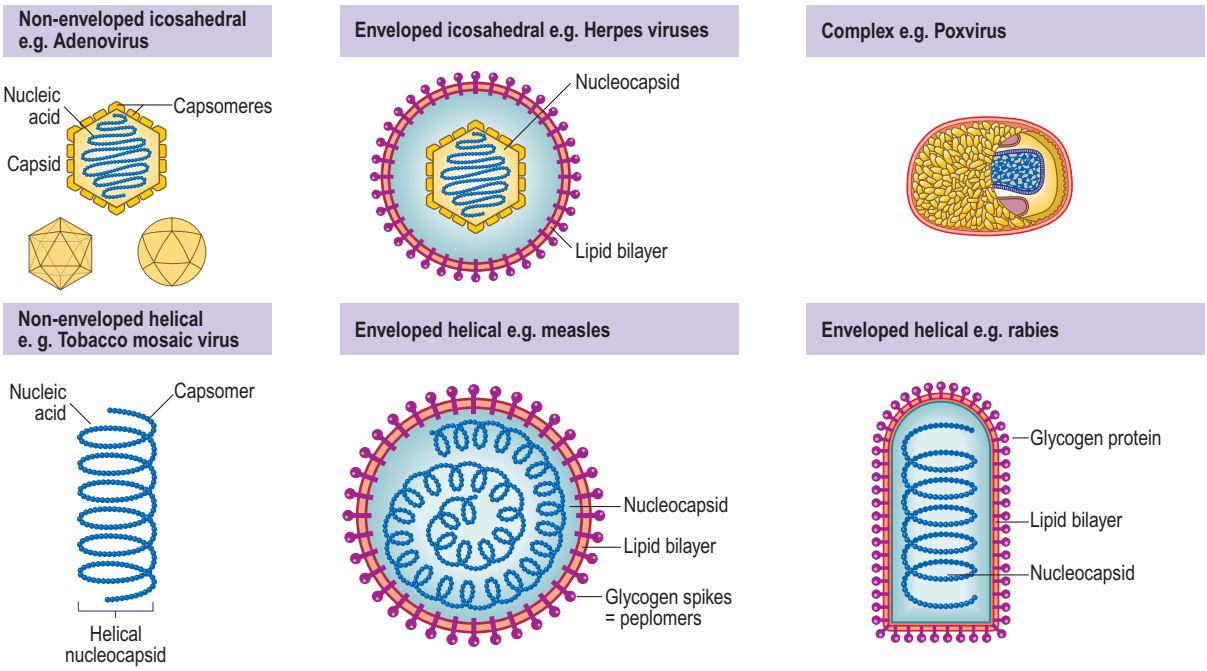


Fig. 2 **Basic types of viral symmetry.**

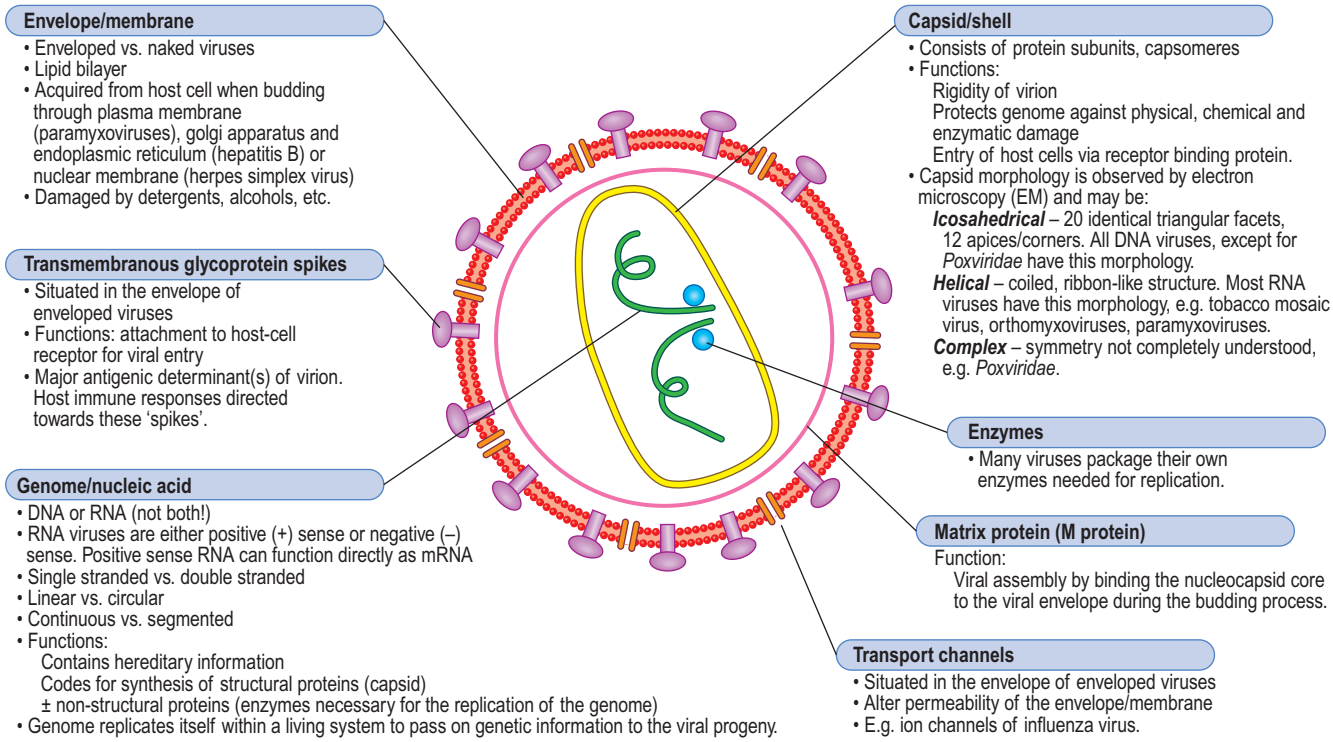


Fig. 3 **Virus structure.**

domains. This also doesn't correspond with the genetic evidence. In fact, there is evidence of viral infections, in the form of entry into cellular genomes, in the earliest forms of cellular life.

Life most likely began in RNA form, and developed into cell-like environments. Within this RNA realm, the **last universal common ancestor** (LUCA) of all modern cellular life existed, and most likely RNA viruses existed alongside LUCA in this RNA realm. Regression and escape hypotheses can be presented within this realm, prior to LUCA, and prior to the development of DNA, which limited rapid diversification due to its greater stability. By the time modern cells of the three domains first appeared, viruses were already part of their environment.

The first proof, however, of the existence of viruses came in 1892 and again six years later, when a Russian scientist, Dmitri Ivanovski and a Dutch botanist, Martinus Beijerinck conducted experiments on tobacco mosaic disease, a disease affecting plants. During these experiments it came to light that the culprits in the 'contagious living fluid' were not bacteria (or their toxins), but even smaller agents that were able to pass through filters (Table 1). These filterable agents were named viruses, which in Latin translates to poison.

A virus can be defined as a small, organised association of macromolecules that is dependent on a living system for growth and replication purposes and mimiviruses. Viruses can be seen as parasites in need of suitable host cells to sustain their life cycles. Viruses are small and sizes range from 20 to approximately 400 nm (Fig. 1). Except for poxviruses and mimiviruses, viruses cannot be visualized by light microscopy (with resolution of >0.25 µm).

Viruses can infect almost all forms of life, including vertebrates, invertebrates, fungi, plants and even bacteria. The susceptibility of host cells to specific viruses depends largely on the presence of specific host cell receptor binding proteins on viral surfaces, as well as on the appropriate cellular machinery needed for viral replication within the host cells.

The basic components of a virus include a genome (consisting of the nucleic acid; DNA **or** alternatively RNA) and a protein shell (capsid). A surrounding membrane or envelope is present in some viruses (which they obtain when budding through host cell membranes) (Fig. 2). Some viruses also encode for and package their own enzymes, which are needed in the replication of their genomes (Fig. 3).

Replication of viruses

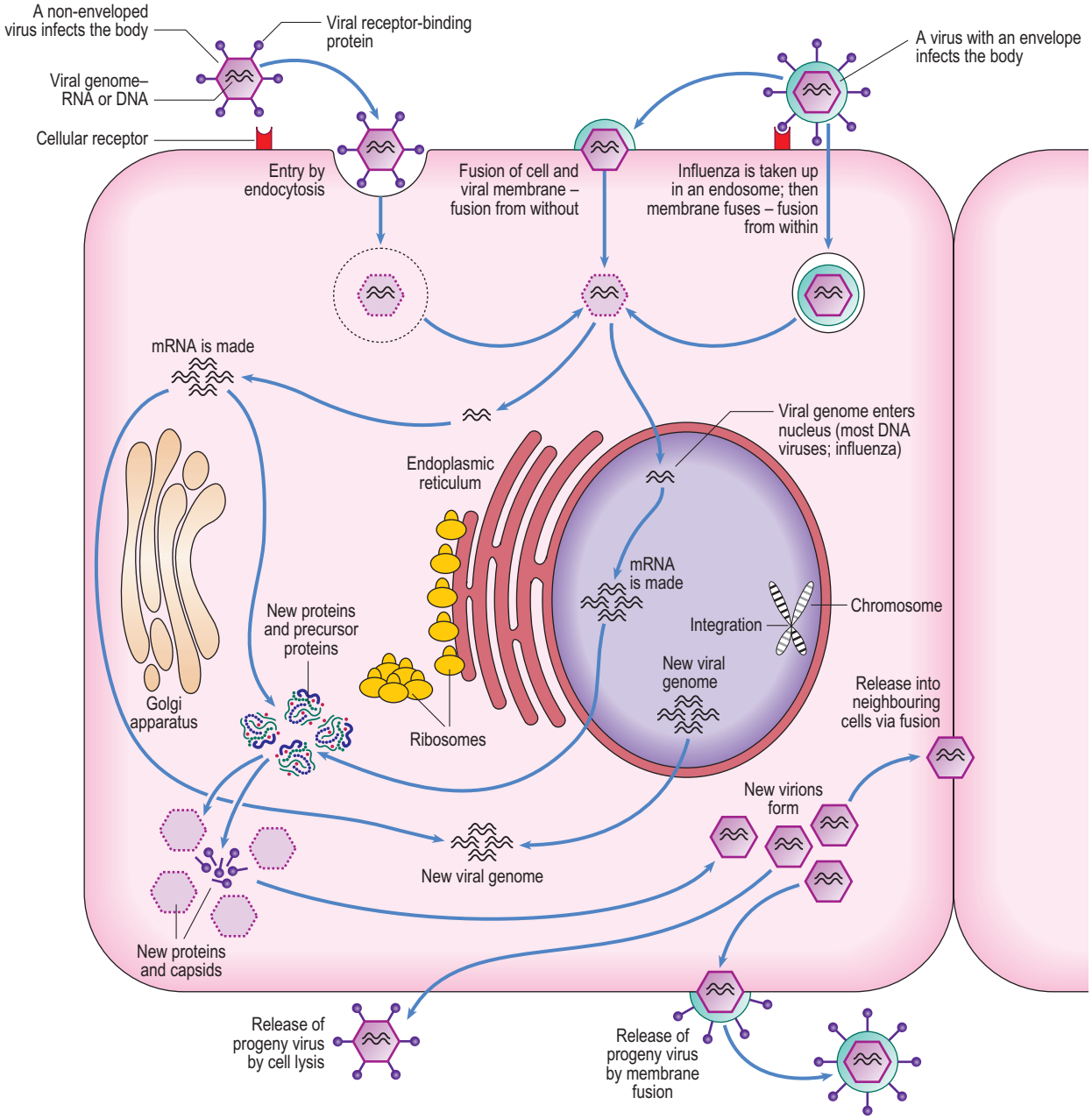


Fig. 1 **Diagram of a cell indicating possible pathways various viruses may use during their replication cycle – entry, uncoating, nucleic acid synthesis, protein synthesis, virion assembly and release from the cell.**

Viruses, as with other organisms, need to replicate in order to continue their infection cycle and spread to new hosts (Fig. 1). For all viruses, a living cell is required to provide at least part of the machinery needed for this process.

The steps in the viral replication cycle are as follows: **attachment** to primary, and sometimes secondary, receptors on the cell; cell **entry** or **penetration** by membrane fusion or receptor-mediated endocytosis (**viropexis**); **uncoating** with release of the genome; **transcription** of the genome into RNA or DNA; transcription of mRNA; **translation** of non-structural and structural proteins; incorporation of the new genomes into new virions during **assembly**; and **release** from the cell by budding, cell lysis, or direct cell-to-cell spread.

In the diagrams in Figure 2, extra steps can be seen that depend on the type of virus infecting the cell. Viruses, such as adenovirus or influenza, which enter cells by means of endocytosis must be released from the endosome. Some viruses encode a genome that can be used directly as mRNA, and early proteins are translated that allow for further steps in replication, and therefore do not need to incorporate polymerase enzymes into their virions – e.g. enteroviruses. Other viruses, such as herpesviruses, have a complex cascade of immediate early (IE), early (E) and late (L) genes that are transcribed and translated prior to further steps in the replication of the genome. These steps can be enhanced

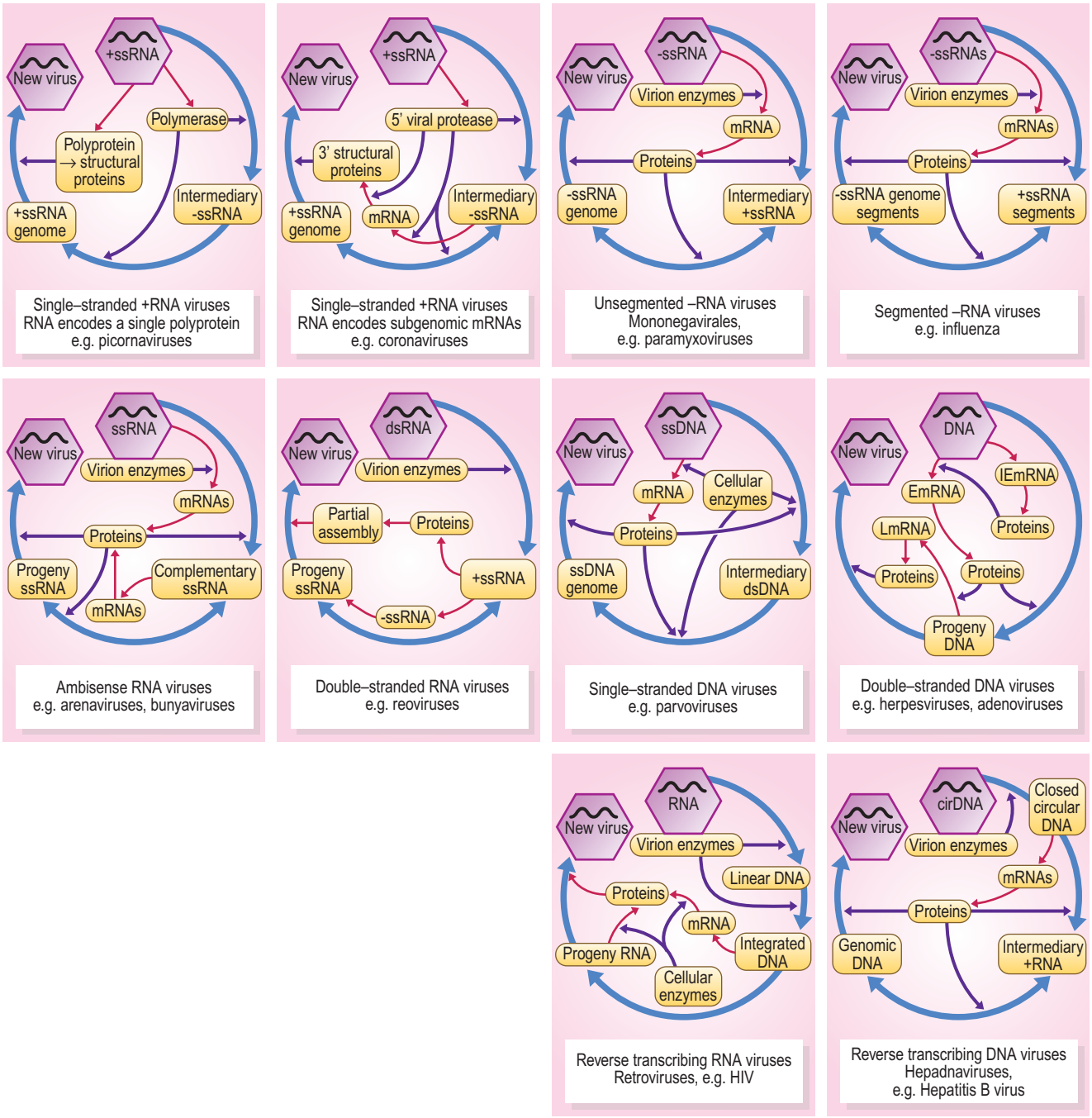


Fig. 2 **Ten different replication strategies for different groups of viruses.**

or inhibited by various gene products to optimize viral replication. Retroviruses include **integration** into the host genome as part of their replication cycle. Some viruses, e.g. most DNA viruses, need to replicate in the nucleus, while most RNA viruses replicate in the cytoplasm.

Processing of mRNA may be as simple as the translation of a **polyprotein** that has auto-cleaving properties, or may include complex strategies such as splicing, multiple stages of mRNA formation under control of other viral gene products, overlapping **reading frames** that produce different proteins from the same area in the genome, and **frame shifting**, where the ribosome shifts into a different reading frame due to signals found part of the way into a gene.

Upon infection, the viral replication cycle enters an **eclipse** phase, where physical identity and infectivity are usually lost, and replication is active on an intracellular molecular level. After this initial local replication, viruses are released from the cells and can infect further cells or be spread to other hosts. This stage is known as the **productive** phase.

Key points

- The basic steps in the replication cycle are: cellular attachment, cell entry, uncoating, transcription of the genome, translation of new proteins, assembly and release from the cell.
- Viruses require cellular enzymes from the host cell in order to replicate.
- Different methods of replication have different implications for pathogenesis and treatment.
- During the eclipse phase, the patient is asymptomatic, and may not be aware of infection.

Classification of viruses

The future of classification

Traditional classification

Traditionally viruses have been classified phenotypically, i.e. by appearance, by size, by genome type, by replication strategy, by host, and by diseases caused. There are two main classification systems in use – the International Committee on Taxonomy of Viruses (ICTV) classification and the Baltimore classification system.

The Baltimore classification divides viruses into seven groups based on a combination of genome type and replication strategy, specifically how mRNA is formed from the original genome. Further subdivision within this system is possible, and can be seen in the ten diagrams showing different replication cycles in Chapter 2, Figure 2.

The ICTV classification is similar to the classification of cellular organisms into genera and species. ICTV uses orders (*-virales*), families (*viridae*), subfamilies (*-virinae*), genera (*-virus*), and species. Relationships are determined by both genome type and sequence similarity. Since the combination of genome type, replication strategy, presence of an envelope, and shape often correlate with genetic relationships, there remains significant overlap between classification systems. The viruses more relevant to humans have been listed in Table 2.

The nucleotide sequence of the viral genome is able to provide information about genetic relationships between viruses, indicating origins and evolution. This is well-studied for the relationship between human immunodeficiency virus (HIV) and its simian counterpart, SIV. The genetic and likely evolutionary relationships (Fig. 1) between viruses are now playing a significant role in classifying viruses and their subtypes. The DNA sequence (Fig. 2) is determined and compared with that of other similar viruses, and a relationship is thus defined. A group of viruses more closely related than the rest will cluster together in a **clade**, with a common ancestral **node**. Depending on how different this clade is from the other related viruses, it may be classified as a separate species or viral subtype (Table 1).

Key points

- Organisms, including viruses, are now being classified more by their genetic sequence and less by their phenotypic appearance (Fig. 3).

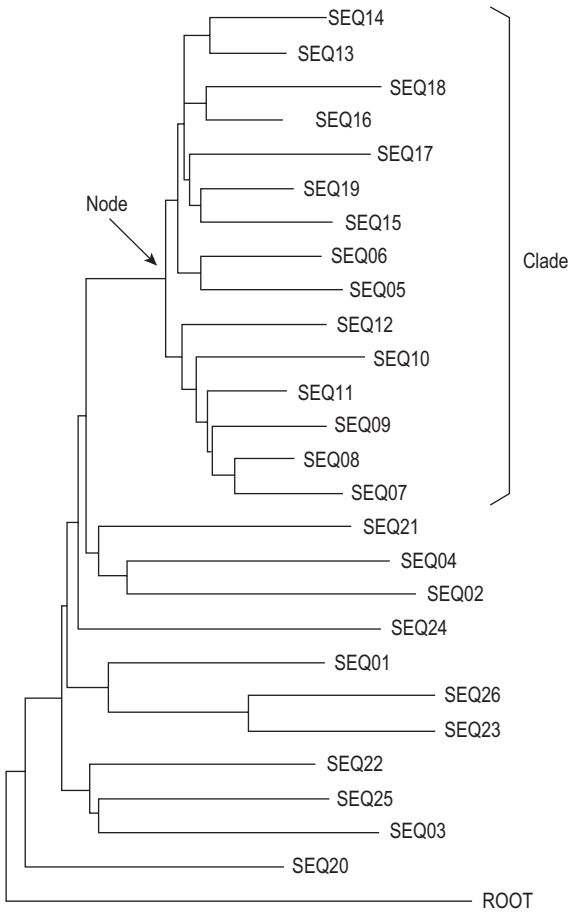


Fig. 1 **Phylogenetic classification.**

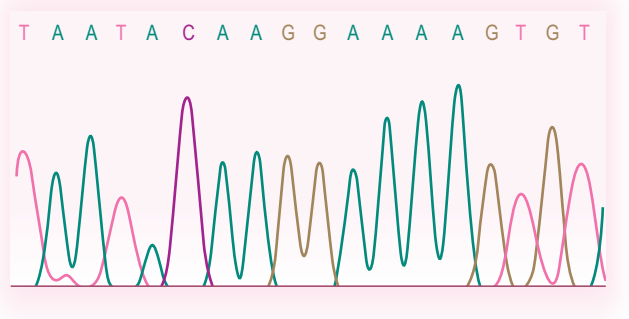


Fig. 2 **DNA sequence.**

Table 1 **Classification of viruses** Historically, viruses have been classified according to a combination of properties:

Type of nucleic acid	DNA or RNA genome, reverse transcription, single- or double-stranded genome, circular or linear genome
Morphology	Envelope present/absent, icosahedral/helical/complex symmetry, size, number of capsomers, surface structure
Epidemiology	Geographic distribution, seasonal spread, age groups, types of transmission, e.g. sexual, perinatal, respiratory
Diseases and pathology caused	Hepatitis, respiratory viruses; inclusion bodies and syncytia
Antigenic properties	Neutralization by certain antibodies – viral typing/subtyping
Sensitivity to various agents	Ultraviolet light, chlorine, ether, formaldehyde, etc.

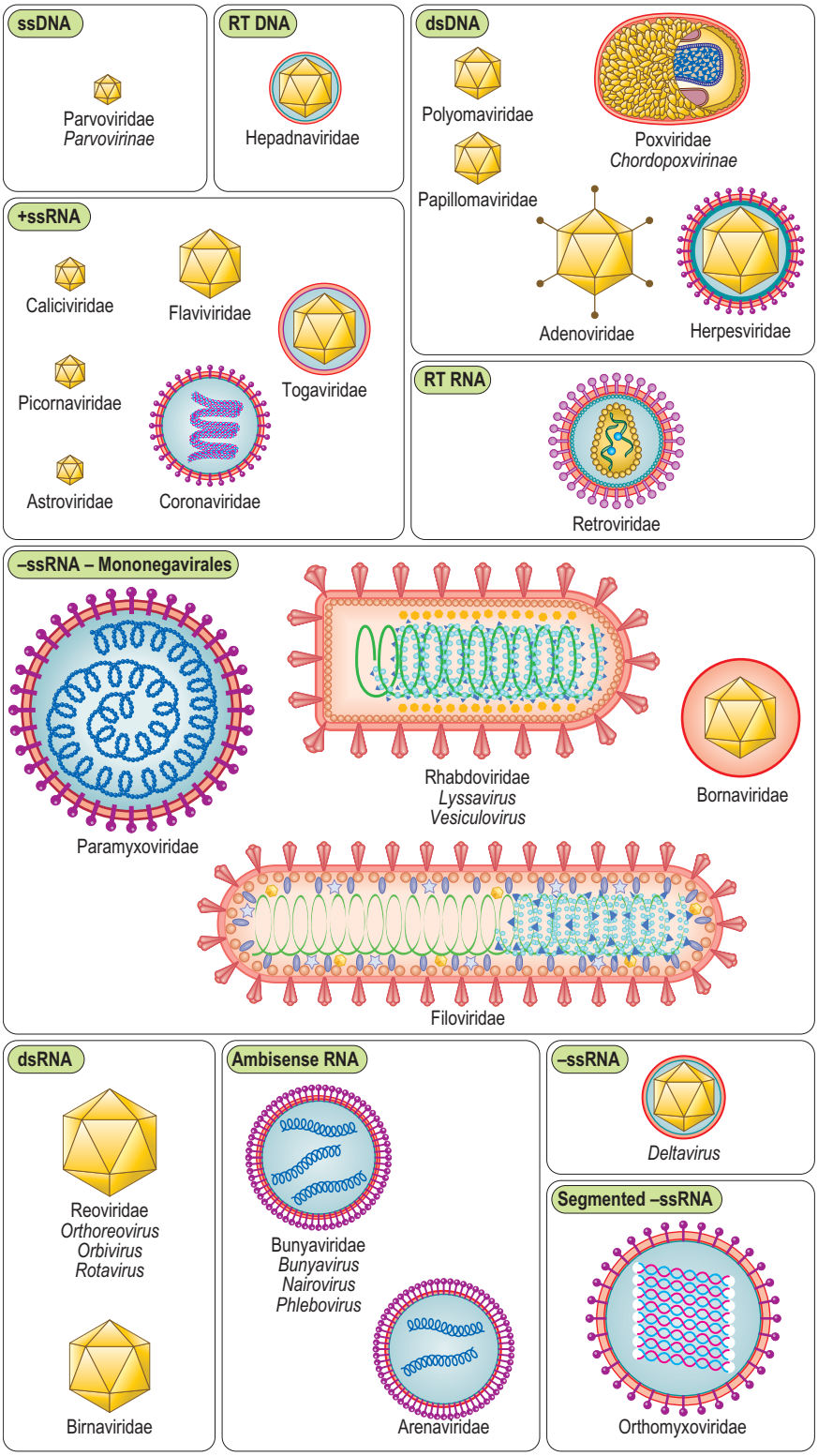


Fig. 3 **Viruses and virus families grouped by their genome type.**

Classification of viruses – List of important virus groups			
DNA viruses			
Family	**Subfamily**	**Genus**	**Virus species**
Single–stranded DNA viruses			
Order: not classified			
Parvoviridae	Parvovirinae	Erythrovirus	Parvovirus B19
		Bocavirus	Human bocavirus
Double–stranded DNA viruses			
Order: Herpesvirales			
Herpesviridae	Alphaherpesvirinae	Simplexvirus	Human herpesvirus 1 (HHV1) (Herpes simplex virus 1)
			HHV2 (Herpes simplex virus 2)
		Varicellovirus	HHV3 (Varicella–zoster virus)
	Betaherpesvirinae	Cytomegalovirus	HHV5 (Cytomegalovirus)
		Roseolovirus	HHV6
			HHV7
	Gammaherpesvirinae	Lymphocryptovirus	HHV4 (Epstein–Barr virus)
		Rhadinovirus	HHV8 (Kaposi's sarcoma–associated herpesvirus)
Order: not classified			
Poxviridae	Chordopoxvirinae	Orthopoxvirus	Variola virus
			Vaccinia virus
			Cowpox virus
			Monkeypox virus
		Parapoxvirus	Orf virus
		Molluscipoxvirus	Molluscum contagiosum virus
		Yatapoxvirus	Yabapox monkey tumour virus
			Tanapox
Adenoviridae		Mastadenovirus	Human adenovirus A: – 12,18,31
			Human adenovirus B: 3,7,11,14,16, 21,34–35,50,55
			Human adenovirus C: 1–2,5–6
			Human adenovirus D: 8–10,13,15,17, 19,20,22–30,32–33,36–39,42–49, 51,53–54
			Human adenovirus E: 4
			Human adenovirus F: 40,41
			Human adenovirus G: 52
Polyomaviridae		Polyomavirus	BK polyomavirus
			JC polyomavirus
Papillomaviridae		Alphapapillomavirus	Human papillomavirus (HPV): 2,6,7, 10,16,18,26,32,34,53,54,61,71, cand90
		Betapapillomavirus	HPV: 5,9,49,cand92,cand96
		Gammapapillomavirus	HPV: 4,48,50,60,88
		Mupapillomavirus	HPV: 1,63
		Nupapillomavirus	HPV: 41
Double–stranded DNA reverse–transcribing viruses			
Order: not classified			
Hepadnaviridae		Orthohepadnavirus	Hepatitis B virus A–H

Classification of viruses – List of important virus groups			
RNA viruses			
Family	**Subfamily**	**Genus**	**Virus species**
Negative–sense single–stranded RNA viruses			
Unsegmented linear genomes			
Order: Mononegavirales			
Bornaviridae		Bornavirus	Borna disease virus
Rhabdoviridae		Lyssavirus	Rabies virus
			Aravan virus
			Australian bat lyssavirus
			Duvenhage virus
			European bat lyssavirus 1
			European bat lyssavirus 2
			Irkut virus
			Khujand virus
			Lagos bat virus
			Mokola virus
			West Caucasian bat virus
Filoviridae		Marburgvirus	Lake Victoria marburgvirus
		Ebolavirus	Reston ebolavirus
			Sudan ebolavirus
			Tai Forest ebolavirus
			Zaire ebolavirus
Paramyxoviridae	Paramyxovirinae	Respirovirus	Human parainfluenza virus 1
			Human parainfluenza virus 3
		Morbillivirus	Measles virus
			Canine distemper virus
			Rinderpest virus
		Rubulavirus	Mumps
			Human parainfluenza virus 2
			Human parainfluenza virus 4
		Henipavirus	Hendra virus
			Nipah virus
		Avulavirus	Newcastle disease virus
	Pneumovirinae	Pneumovirus	Human respiratory syncytial virus
		Metapneumovirus	Human metapneumovirus
Segmented linear genomes			
Order: not classified			
Orthomyxoviridae		Influenzavirus A	Influenza A virus
		Influenzavirus B	Influenza B virus
		Influenzavirus C	Influenza C virus
Arenaviridae		Arenavirus	Lassa virus
			Chapare virus
			Guanarito virus
			Ippy virus
			Junin virus
			Lassa virus
			Lulo virus
			Lymphocytic choriomeningitis virus
			Machupo virus
			Mobala virus
			Mopela virus
			Sabiá virus

Table 2

Classification of viruses – List of important virus groups			
RNA viruses			
Family	**Subfamily**	**Genus**	**Virus species**
Bunyaviridae		Orthobunyavirus	Bunyamwera virus
			California encephalitis virus
		Hantavirus	Hantaan virus
			Puumala virus
			Sin Nombre virus
		Nairovirus	Crimean–Congo haemorrhagic fever virus
		Phlebovirus	Rift Valley Fever virus
Circular genomes			
Order: not classified			
		Deltavirus	Hepatitis delta virus (Hepatitis D)
Positive–sense single–stranded RNA viruses			
Order: Picornavirales			
Picornaviridae		Enterovirus	Human enterovirus A
			Human enterovirus 71
			Human coxsackievirus A: 2–8,10,12,14,16
			Human enterovirus B
			Human coxsackievirus B1–6
			Human coxsackievirus A9
			Human enterovirus 69
			Human echovirus 1–7,9,11–21,24–27,29–33
			Human enterovirus C
			Human coxsackievirus A1: 1,11,13,15,17–22,24
			Poliovirus 1-3
			Human enterovirus D
			Human enterovirus 68,70
			Human enterovirus E
			Human rhinovirus A, B, C (3 species)
		Cardiovirus	Encephalomyocarditis virus
		Aphthovirus	Foot–and–mouth disease virus
		Hepatovirus	Hepatitis A virus
		Parechovirus	Human parechovirus
			Liungan virus
Order: not classified			
Caliciviridae		Norovirus	Norwalk virus:
			Norwalk virus
			Desert Shield virus
			Lordsdale virus
			Mexico virus
			Southampton virus
			Hawaii virus
			Snow Mountain virus
		Sapovirus	Sapporo virus
Astroviridae		Mamastrovirus	Human astrovirus
Hepeviridae		Hepevirus	Hepatitis E virus

Classification of viruses – List of important virus groups			
RNA viruses			
Family	**Subfamily**	**Genus**	**Virus species**
Positive–sense single–stranded RNA viruses			
Order: not classified			
Flaviviridae		Flavivirus	Kyasanur Forest disease virus
			Omsk haemorrhagic fever virus
			Dengue virus
			Japanese encephalitis virus
			St. Louis encephalitis virus
			West Nile virus
			Yellow fever virus
		Hepacivirus	Hepatitis C virus
Togaviridae		Alphavirus	Chikungunya virus
			Middelburg virus
			O'nyong–nyong virus
			Ross River virus
			Semliki Forest virus
			Sindbis virus
			Eastern equine encephalitis virus
			Venezuelan equine encephalitis virus
			Western equine encephalitis virus
		Rubivirus	Rubella virus
Order: Nidovirales			
Coronaviridae	Coronavirinae	Alphacoronavirus	Human coronavirus 229E
			Human coronavirus NL63
			Human coronavirus HKU1
			Severe acute respiratory syndrome coronavirus
	Torovirinae	Torovirus	Human torovirus
Single–stranded RNA reverse transcribing viruses			
Order: not classified			
Retroviridae		Deltaretrovirus	Primate T–lymphotropic virus 1 (HTLV–1)
			Primate T–lymphotropic virus 2 (HTLV–2)
		Lentivirus	Human immunodeficiency virus 1
			Group M: subtypes A1,A2,B,C,D,F1,F2,G,H,J,K, circulating recombinant forms, (CRF) 1–48
			Group N
			Group O
			Group P
			Human immunodeficiency virus 2
			Groups A–G, one CRF
Double–stranded RNA viruses			
Order: not classified			
Reoviridae	Sedoreovirinae	Rotavirus	Rotavirus A–E
	Spinarreovirinae	Coltivirus	Colorado tick–fever virus

Table 2, cont'd

Viruses and their system/hosts

Table 1 **Clinical associations with the viruses significant to human medicine**

Virus or virus type	Some of the clinical associations
Adenoviruses	Conjunctivitis, keratitis, otitis media, rhinitis, common cold, pharyngitis, pneumonia/pneumonitis (adults and infants), meningitis, encephalitis/meningoencephalitis, gastritis, gastroenteritis, intussusception, myocarditis, nephritis, haemorrhagic cystitis, hepatitis
Arboviruses	Meningitis, encephalitis, meningoencephalitis, arthritis, arthralgia, rash, haemorrhage
Astroviruses	Gastroenteritis
BK virus	Persisting renal infection, post-transplant nephropathy, haemorrhagic cystitis
Caliciviruses	Gastroenteritis
Cytomegalovirus	Cataracts (congenital), retinitis, tonsillitis, parotitis, meningitis, encephalitis, meningoencephalitis, transverse myelitis, pneumonia, pneumonitis in infants, oesophagitis, haemorrhagic enteritis/colitis, proctitis, acute hepatitis, hepatosplenomegally, pancreatitis, nephritis, persisting renal infection, thromocytopenia, pancytopenia, atypical mononuclear cells, localised lymphadenopathy, splenomegally, immunsuppression, sexually transmitted infections, congenital defects, intrauterine damage, neonatal jaundice, intrauterine infection, breast milk transmission, polyradiculoneuritis (post-infectious), adrenalitis, inner ear defects
Coronaviruses	Rhinitis, common cold, bronchitis, bronchiolitis, pneumonia, pneumonitis, severe acute respiratory syndrome (SARS)
Crimean–Congo haemorrhagic fever	Haemorrhagic fever, rash
Dengue haemorrhagic fever	Erythematous exanthem/enanthem, arthritis, arthralgia, haemorrhagic fever
Epstein-Barr virus	Lacrimal glands and ducts, conjunctivitis, retinitis, ophthalmoplegia, nasopharyngeal carcinoma, tonsillitis, meningitis, myelitis, myocarditis, acute hepatitis, anaemia, pancytopenia, atypical mononuclear cells, localised lymphadenopathy, splenomegally, Burkitt's lymphoma, intracerebral lymphomas, polyradiculoneuritis (post-infectious)
Enteroviruses	Lacrimal gland and duct infection, retinitis, gingivostomatitis, conjunctivitis, rhinitis, otitis media, parotitis, meningitis, encephalitis, meningoencephalitis, myelitis, poliomyelitis, common cold, laryngitis, pharyngitis, bronchitis, bronchiolitis, pneumonia/pneumonitis in infants, pleurodynia, erythematous exanthem/enanthem, hand foot and mouth disease, herpangina, myalgia, myositis, myocarditis, pericarditis, enteritis, leukopenia, lymphopenia, atypical mononuclear cells, intrauterine infection, intrauterine damage, neonatal sepsis, adrenalitis
Filoviruses	Pharyngitis, adrenalitis, oral enanthem, myelitis, erythematous exanthem/enanthem, desquamation, arthritis, arthralgia, bradycardia, splenomegally, haemorrhagic fever, hydrops fetalis
Hantaviruses	Meningitis, acute respiratory syndromes, arthritis, arthralgia, myalgia/myositis, myocarditis, nephritis, haemorrhagic fever
Hepatitis A	Acute hepatitis, myalgia/myositis
Hepatitis B	Acute hepatitis, chronic hepatitis, cirrhosis, hepatocellular carcinoma, arthritis, arthralgia, sexually transmitted infection, peripartum infection, neonatal jaundice, intrauterine infection, glomerulonephritis, vasculitis
Hepatitis C	Acute hepatitis, chronic hepatitis, petechiae/purpura, cirrhosis, hepatocellular carcinoma, cold agglutanins, sexually transmitted infection, glomerulonephritis, neonatal jaundice, intrauterine infection, ocular manifestations, porphyria cutanea tarda
Hepatitis D	Acute hepatitis, chronic hepatitis, cirrhosis
Hepatitis E	Acute hepatitis, neonatal jaundice, chronic hepatitis
HHV6, HHV7	Meningitis, erythematous exanthem/enanthem
HHV8	Kaposi's sarcoma, body cavity lymphoma, primary effusion lymphoma (PEL), Castleman's syndrome
HIV	Immunosuppression, sexually transmitted infection, intrauterine infection, peripartum infection, breast milk transmission, retinitis, ophthalmoplegia, central paresis, peripheral paresis, erythematous exanthem/enanthem, seborrheic eczema, myalgia/myositis, oesophagitis, leukopenia, lymphopenia, generalised lymphadenopathy, tonsillitis, meningitis, encephalitis, meningoencephalitis, chronic encephalitis/encephalopathy, myelitis, polyradiculoneuritis (post-infectious)
Human papillomavirus	Warts, benign tumours, malignant tumours, cervix carcinoma, vulva carcinoma, penile carcinoma, epidermodysplasia verruciformis, oral papillomas, oropharyngeal carcinoma
Herpes simplex virus	Urethritis, herpes labialis, herpes genitalis, vesicles, polyradiculoneuritis (post-infectious), lacrimal gland and duct infection, conjunctivitis, keratitis, acute necrotising retinitis, uveitis, gingivostomatitis, meningitis, encephalitis, meningoencephalitis, transverse myelitis, pneumonia, pneumonitis in infants, eczema herpeticum, oesophagitis, proctitis, acute hepatitis, prostatitis, vesicular disease, congenital defects, neonatal jaundice, neonatal sepsis, intrauterine infection, peripartum infection
Human bocavirus	Bronchitis, bronchiolitis, pneumonia, pneumonitis in infants
Human metapneumovirus	Tracheitis, tracheobronchitis, pneumonia, pneumonitis in infants, common cold
Human T-cell lymphotropic virus	Adult T-cell leukaemia, tropical spastic paraparesis, encephalitis, meningoencephalitis, myelopathy, peripheral paresis, T-cell lymphoma, generalised lymphadenopathy, sexually transmitted infection, intrauterine infection
Influenza	Conjunctivitis, pharyngitis, laryngitis, tracheitis, tracheobronchitis, bronchitis, bronchiolitis, pneumonia, pneumonitis, pericarditis, otitis media, polyradiculoneuritis (post-infectious), common cold, Reye's syndrome, myalgia/myositis
JC virus	Chronic encephalitis/encephalopathy, persisting renal infection
Lassa virus	Haemorrhagic fever, encephalitis, meningoencephalitis, pericarditis, pharyngitis, intrauterine damage, inner ear defects
Lymphocytic choriomeningitis virus	Meningoencephalitis, leukopenia, thrombocytopenia
Measles	Pharyngitis, erythematous exanthem/enanthem, desquamation, tracheitis, tracheobronchitis, bronchitis, bronchiolitis, pneumonia, pneumonitis in infants, conjunctivitis, keratitis, otitis media, oral enanthem, meningitis, encephalitis, meningoencephalitis, chronic encephalitis/encephalopathy, lacrimal glands and ducts, vasculitis, leukopenia, lymphopenia, immunosuppression
Molluscipox virus	Molluscum contagiosum, conjunctivitis
Mumps	Parotitis, orchitis/oophoritis (adnexitis), lacrimal gland and duct infection, conjunctivitis, keratitis, inner ear defects, thyroiditis, meningitis, facial paresis, arthritis, arthralgia, myocarditis, pancreatitis, splenomegally, polyradiculoneuritis (post-infectious)
Orfvirus	Papules
Parainfluenza	Conjunctivitis, otitis media, common cold, laryngitis, pharyngitis, tracheitis, tracheobronchitis, bronchitis, bronchiolitis, pneumonia, pneumonitis in infants
Parvovirus B19	Erythematous exanthem/enanthem, arthritis, arthralgia, anaemia, hydrops fetalis, intrauterine infection, myocarditis, vasculitis, pancytopenia, atypical mononuclear cells
Polioviruses	Poliomyelitis, ophthalmoplegia, encephalitis, meningoencephalitis, central paresis, arthritis, arthralgia, post-poliomyelitis syndrome
Prions	Chronic encephalitis/encephalopathy

Table 1 **Clinical associations with the viruses significant to human medicine—cont'd**

Virus or virus type	Some of the clinical associations
Rabies	Encephalitis, meningoencephalitis, ophthalmoplegia
Respiratory syncytial virus	Otitis media, rhinitis, common cold, laryngitis, pharyngitis, tracheitis, tracheobronchitis, bronchitis, bronchiolitis, pneumonia/pneumonitis in infants and elderly
Rhinoviruses	Rhinitis, common cold, bronchitis, bronchiolitis, pneumonia/pneumonitis in infants
Rift Valley Fever	Haemorrhagic fever, retinitis
Rotavirus	Gastroenteritis, intussusception
Rubella	Erythematous exanthem/enanthem, localised lymphadenopathy, keratitis, cataracts (congenital), congenital glaucoma, meningitis, chronic encephalitis/encephalopathy, pharyngitis, arthritis, arthralgia, congenital heart defects, other congenital defects, intrauterine infection, inner ear defects, arthritis, arthralgia
Yellow fever	Haemorrhagic fever, acute hepatitis, arthritis, arthralgia, bradycardia
Varicella	Vesicular disease, pneumonia/pneumonitis in infants and adults, zoster oticus, congenital defects, intrauterine damage, neonatal jaundice, neonatal sepsis, intrauterine infection, peripartum infection, lacrimal glands and ducts, conjunctivitis, keratitis, cataracts (congenital), acute necrotising retinitis, ophthalmoplegia, meningitis, encephalitis, meningoencephalitis, Reye's syndrome, transverse myelitis, facial paresis, arthritis, arthralgia
Viral haemorrhagic fevers	Haemorrhage, petechiae/purpura, myalgia/myositis, leukopenia, lymphopenia, thrombocytopenia, generalized lymphadenopathy

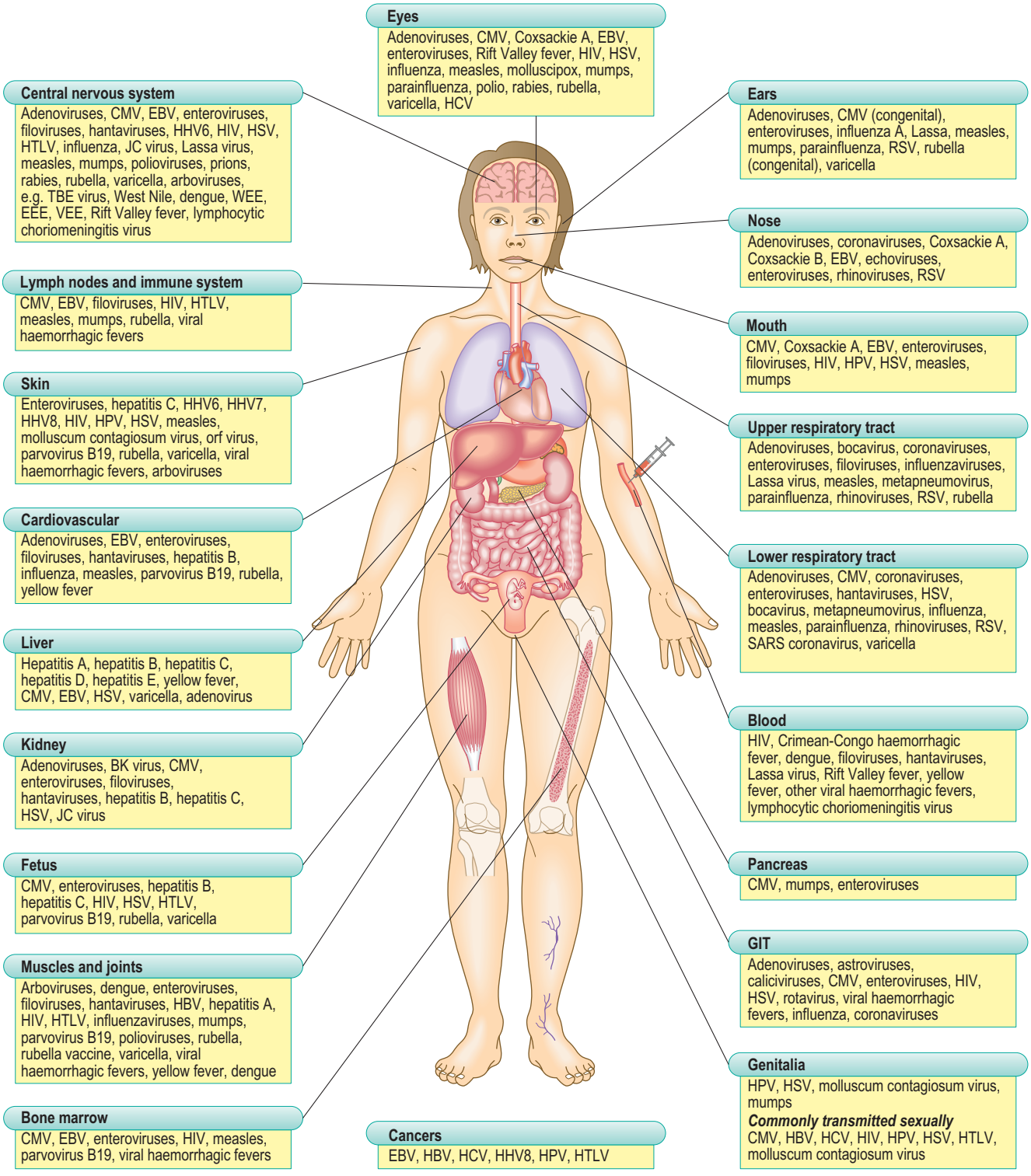


Fig. 1 Diagram of the human body showing organs and systems along with the viruses that affect them.

Virus transmission

Viruses need to be able to spread from one host to another in order to survive. Virus properties influence the way they are transmitted. Some viruses such as rotavirus, hepatitis A and enteroviruses, secreted in human faeces, are very resistant and can survive for many weeks in the environment. Other viruses such as HIV and many paramyxoviruses are quickly inactivated by drying out and need close contact between humans to be transmitted. The second factor influencing transmission mode is the place where the virus replicates and the amount of virus in a specific compartment: HIV is found in blood and lymphoid tissue and genital secretions, whereas paramyxoviruses are found in respiratory secretions and droplets – thus explaining their different routes of transmission. The third factor comprises the specific adaptations which allow viruses to reach the entry site or site of initial replication. Viruses excreted in faeces are often ingested again when people eat contaminated food, eat with contaminated hands or drink contaminated water. These viruses have thick coats (capsids) which allow them to survive the very acidic stomach and alkaline bile to reach the small intestine where they infect enterocytes in order to replicate.

Another example is the influenza virus which has an enzyme on its surface – neuraminidase – that can break up the receptors on respiratory cells that could hold viruses onto the mother cell after they have budded out. This enzyme may also help to break up mucus that could prevent the virus from spreading through the respiratory tract.

Viruses that are adapted for a part of their life cycle in a vertebrate host and another part in an arthropod are called arboviruses. These viruses replicate in both the arthropod host (usually in the salivary glands) and the vertebrate host with the arthropods (insects or arachnids such as ticks) transmitting these viruses from the one vertebrate host to the other. Some arboviruses are also transmitted by the arthropods to their eggs (transovarial transmission). These viruses are thus adapted to replicate in very different conditions and often infect a large range of hosts.

There are many different routes of transmission, which are outlined in Table 1. The main transmission routes are human-to-human transmission, e.g. by droplets through coughing (Fig. 1A) and sneezing (Fig. 1B); mother-to-child (vertical) transmission (Fig. 2) and blood-borne transmission (Fig. 3), which are special cases of human-to-human transmission; human-to-environment-to-human, e.g. faeco-oral transmission (Fig. 4); animal-to-human: direct, e.g. dog bite (Fig. 5) or animal/human- via a vector-to-human (Fig. 6).

Table 1 **Routes of virus transmission of viruses to humans**

Route	Nature of transmission	Examples
Human-to-human transmission	Close contact: kissing	Oral herpes simplex and Epstein-Barr virus
	Large respiratory droplets	Paramyxoviruses (mumps), rubella
	Blood and blood products	HIV, Hepatitis B virus, Hepatitis C virus
	Sexual contact	Genital herpes, HIV, genital warts
	Abrasions	Skin warts, molluscum contagiosum
	Contaminated hands – virus excreted in faeces	Hepatitis A virus, rotavirus
	Contaminated hands – virus in respiratory secretions	Rhinovirus
Vertical transmission (mother-to-child)	Intrauterine	Rubella, HIV
	During the birth process	Hepatitis B virus, HIV
	Breastfeeding	HIV, HTLV
Human-to-environment-to-human	Virus excreted in faeces: contaminated food, surfaces, water	Hepatitis A virus, enteroviruses, rotavirus, caliciviruses
	Contaminated surfaces respiratory secretions	Rhinovirus
	aerosolised virus from respiratory secretions	Influenza
Animal-human via a vector to human: arboviruses	Mosquito-borne	Yellow fever, West Nile
	Tick-borne	Tick borne encephalitis, Crimean–Congo haemorrhagic fever
Direct animal-to-human	Animal bites	Rabies

Fig. 1 **Virus transmission by (A) coughing (B) sneezing.**

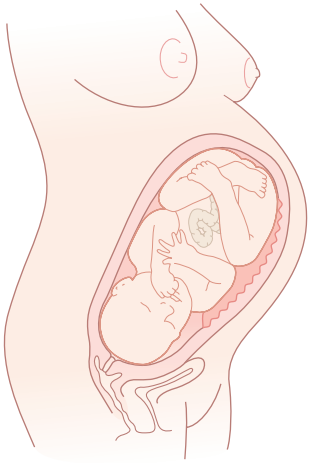


Fig. 2 **Intrauterine mother-to-child (vertical) transmission.**

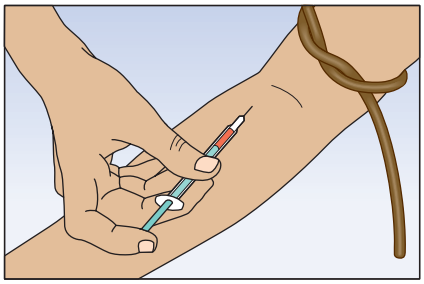


Fig. 3 **Human-to-human transmission of viruses by blood.**

Fig. 5 **Human-to-environment-to-human transmission, e.g. Hepatitis A via unwashed hands to food.**

Fig. 4 **Animal-to-human transmission directly from a dog, e.g. rabies.**

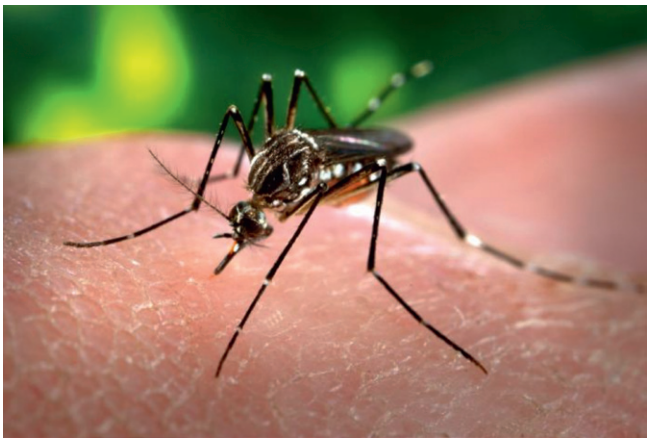


Fig. 6 **Vector-borne transmission via Aedes mosquito** (Photo courtesy of CDC/James Gathany).

STOP THINK

- Which characteristics are necessary for viruses to be effectively transmitted through the environment?
- Does sexual transmission hold a benefit for viral survival?
- Viruses which are transmitted by droplets when coughing or sneezing often end up on surfaces or on the hands of people. People also often touch their own eyes. Considering these factors - what can one do to reduce the risk of contracting these infections?

Key points

- Virus characteristics influence their ability to survive in different environments and therefore their routes of transmission.
- Viruses are transmitted human-to-human, human-to-environment-to-human, animal-to-human or animal/human- via a vector-to-human.
- Some viruses are extremely host specific but arboviruses usually need to be promiscuous in order to survive and to be effectively transmitted.

Susceptibility and resistance to viral disease

It is well-known that people are not equally susceptible to a particular infection. This chapter gives a broad overview of factors that affect susceptibility to viral disease, whereas the role of the immune system in defence against viral infection is discussed in greater detail in the next chapter. There are many factors that can influence both the ease with which somebody can be infected and the severity of the disease should the infection occur. Human beings differ from each other with regards to their genetics, environments, medical history, gender and age. Broadly susceptibility or resistance to infections can be classified as inborn/primary (all the genetic causes) or acquired referring to all the other causes.

Inborn resistance or susceptibility

Genes affect our susceptibility to infections in many ways. Some people have inborn genetic conditions that make them susceptible to a wide range of conditions. This is referred to as congenital immunodeficiency. Certain congenital immunodeficiency states that affect T-cells, antibody production or both are associated strongly with an increased risk of susceptibility to certain viral infections, whereas defects of phagocytes or deficiency of complement are more relevant to other pathogens. The congenital conditions associated with this increased risk are listed in Table 1.

Genetic resistance to specific viral infections

The fact that there are genetic differences between individuals with different allele frequencies in different populations implies that differences in resistance to specific infections exist between individuals and groups. A well-known example involves people who are homozygous for a certain polymorphism in the CCR5 receptor (a co-receptor used by HIV), the Δ32 deletion, are highly resistant to HIV infection (Fig. 1). This polymorphism is especially common in certain European populations. Another example is that people from Haiti and West Africa are resistant to the complications of Dengue virus, an association which is most probably genetic, although the particular polymorphisms have not yet been identified. There are many strains of noroviruses which can cause diarrhoea but any particular norovirus strain only causes diarrhoea in particular patients who have tissue group receptors that allow infection – other individuals are resistant. Host species specific viral resistance can be conferred by cellular restriction factors: Tripartite motif protein 5-alpha (TRIM5α) is an intracellular protein that binds to retroviral capsids and prevents replication, most likely by targeting these viral capsids for intracellular degradation by the proteasome. A particular retrovirus (from the genus lentivirus) and its particular host species' TRIM5α appear to have co-evolved. Therefore a particular TRIM5α would restrict replication of lentiviruses adapted to other host species, whereas its own lentivirus evolved to escape the action of the host's TRIM5α. Other restriction factors, APOBEC3G and APOBEC3F, restrict viral replication by resulting in hypermutation of the single stranded DNA, which is formed during reverse transcription. HIV-1 encoded protein vif has evolved to escape the action of APOBEC3G/F. TRIM5α and APOBEC3G/F therefore confers host species-specific natural resistance to retroviral infections.

Table 1 **Congenital conditions associated with increased susceptibility to viral infections**

Deficit	Conditions	Susceptibility to virus infections
T-cells	Chronic mucocutaneous candidiasis	Herpes simplex, varicella zoster (recurrence)
	DiGeorge syndrome	General increased susceptibility to viral infections
T and B cells	Ataxia telangiectasia	Recurrent respiratory viral infections
	Severe combined immunodeficiency (SCID)	Highly susceptible to most viral infections
	Wiskott-Aldrich syndrome	Respiratory infections, recurrent herpes simplex
	X-linked lymphoproliferative syndrome	Epstein-Barr virus infection leads to life threatening illness
Antibodies (due to B cell deficit)	Common variable immunodeficiency	Respiratory and gastrointestinal infections (such as rotavirus)
	Selective antibody deficiency (IgA)	Prolonged excretion of poliovirus
	X-linked agammaglobulinaemia	Respiratory and gastrointestinal infections (including viral infections)

Infections leading to immunodeficiency

Acquired causes of immunodeficiency can be infective or non-infective. The infective cause that people most commonly think of is HIV, which, without treatment, causes chronic progressive immunodeficiency. HTLV-1

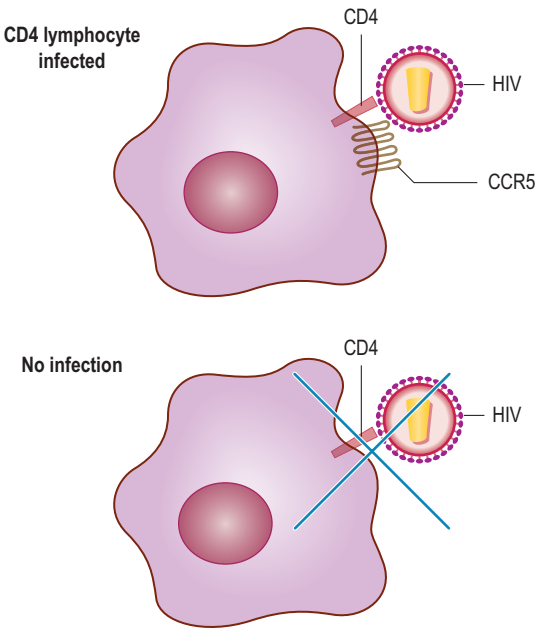


Fig. 1 **Transmitted HIV usually needs the CCR5 co-receptor to infect CD4 lymphocytes. When these receptors are absent due to the Δ32 homozygosity, individuals are highly resistant to HIV infection.**

- What effect does human migration have on susceptibility to infection?
- Why is there such a big variation in susceptibility to infection among humans?

also causes a chronic immunodeficiency by affecting the T-cell populations. Many other viruses can cause a temporary immunodeficiency during the acute stage or in the following weeks, for instance measles virus increases susceptibility to many causes of diarrhoeal and respiratory disease. Other viruses associated with temporary immunodeficiency following an acute infection are Epstein-Barr virus and cytomegalovirus.

Medical history, drugs, nutrients, sunlight, stress and hormones

There are also many non-infectious causes of acquired immunodeficiency which can be related to medical conditions, medical treatment or the environment. Diabetes mellitus is associated with increased susceptibility to certain fungal and bacterial diseases and also with an increased risk of developing severe influenza. Patients with haematological malignancies are more likely to have herpes zoster or progressive multifocal leuco-encephalopathy (PML) than normal individuals. Immunosuppressive treatment to prevent organ rejection in organ transplant patients or patients with allergic, auto-immune or connective tissue diseases can render these patients susceptible to a variety of infections including certain viral infections. A common example is cytomegalovirus disease in renal transplant patients. Nutrition also plays a role in resistance to infection. Vitamin A deficiency has been shown to be associated with severe measles virus infection and an increased risk of corneal damage (keratitis) leading to blindness, whereas megadose vitamin A supplementation can be used to limit the complications of measles. Children with protein energy malnutrition are more susceptible to a variety of conditions including disseminated herpes simplex infection. The supplementation of B vitamins, and vitamins C and E has been associated with a slightly slower progression of HIV in patients both in Tanzania and in the USA. Other environmental factors such as excessive exposure to ultraviolet light from the sun increases the risk of oro-labial reactivation of herpes simplex virus infection, whereas moderate sun exposure resulting in increased vitamin D synthesis may boost resistance to influenza virus and probably other respiratory pathogens. Emotional or physical stress is associated with increased severity of the common cold. Also severe physical exertion is associated with a recrudescence of hepatitis A virus or in an increase in the severity of poliomyelitis. Hormonal changes such as during the menstrual cycle influences reactivation of herpes simplex virus and pregnancy is associated with a high risk of varicella pneumonia and more severe hepatitis E infection.

Age and gender

Neonates are more susceptible to severe systemic disease caused by enteroviruses. Common childhood diseases may lead to severe disease in non-immune adults. Measles virus showed a very high mortality when it was introduced into areas where no adults were immune. Varicella is also more severe in healthy adults than in healthy children. Hepatitis A virus is usually asymptomatic in children under two but can cause severe hepatitis in adults, especially the elderly. Old age also renders people more susceptible to severe respiratory tract infections caused by viruses such as influenza and respiratory syncytial virus (RSV) and it is associated with the reactivation of varicella leading to zoster (shingles).

Gender may affect susceptibility to viruses that are sexually transmitted. For certain viruses such as HTLV-1, which is sexually transmitted, women are much more likely to be infected by their male partners than the other way round. The same also applies to HIV, but to a lesser extent.

Socio-economic conditions and population density

Many viruses are acquired at an earlier age in areas with poor socio-economic conditions or a high population density. Whether this is mainly due to increased exposure or whether physical or emotional stresses render these people more susceptible is unknown.

Breastfeeding

Children, in resource-limited settings, who are breastfed, are less likely to suffer from diarrhoea than bottle-fed children. This is likely to be due to both the increased exposure due to the contamination of bottle feeds in bottle-fed children and the protective role of breast milk factors such as IgA.

The study of resistance and susceptibility

The factors discussed here are but a few of many that help to explain why a certain individual would either be susceptible or resistant to a particular infection or disease. These factors also interact in a complex manner, which makes it difficult to study and quantify risk. The data gathered as part from the Human Genome Project, and large well-planned epidemiological studies and tools, such as epidemiological modelling, can in future help to elucidate many more such resistance and susceptibility factors. Understanding which people are at increased risk of acquiring a particular infection or severe disease is clinically helpful in targeting prevention measures.

Key points

- Genetic factors, age, gender, medical history, current infections, behaviour and environmental factors can all affect susceptibility to a particular viral infection.
- A few factors associated with respective resistance or susceptibility to particular infections are known but the role of many other factors still need to be elucidated.
- The study of susceptibility (or resistance) factors is complex and requires well-planned epidemiological studies.

Mechanisms of antiviral immunity

Non-specific factors

The human body is well adapted to stay healthy or recover back to health after infection. These protective adaptations are all part of a complex system maintaining health. The first layer of defence comprises non-specific factors: the fatty secretions on our skin and substances in tears and saliva have anti-infective properties; mucus on our mucosal surfaces traps viruses and microbes and cilia export them to the throat and nose where they are coughed out; and stomach acid destroys many viruses and bacteria in order to protect the gastro-intestinal tract.

The innate response

The second layer of defence is the innate immune response. This response consists of cells such as neutrophils, macrophages, natural killer cells and substances such as complement and cytokines. Macrophages and dendritic cells, also called antigen presenting cells, take-up and process foreign agents, secrete cytokines that stimulate the innate and adaptive response and present short peptides in association with major histocompatability complex II (MHC II) in order to stimulate T-helper cells. Dendritic cells are thus important in linking the innate and adaptive immune responses since immature dendritic cells are recruited to areas of infection where they take-up and process foreign antigen and then mature and migrate to the lymph nodes where they present antigen to T-helper cells which again orchestrate the adaptive immune response. Natural killer cells are especially important in the early stages of a viral infection, since they recognise virus infected cells non-specifically. This recognition leads to the activation of pathways that stimulate a response destroying infected cells. The innate response also links up to the adaptive immune response via the secretion of cytokines. A variety of cytokines, secreted by cells of the innate and adaptive immune response, act as chemical messengers, stimulating and recruiting helper- and cytotoxic T-lymphocytes to the area of infection. Fig. 1 illustrates important cells in the innate and adaptive immune system and their major roles. Although the innate immune response is not pathogen specific to the same degree as the adaptive immune response, pattern recognition receptors (PRRs) confer some specificity by 'sensing' the presence of pathogen associated molecular patterns (PAMPs). The most well-known are toll-like receptors (TLRs). Different TLRs recognise viral proteins, DNA or RNA, and through complex molecular pathways, initiate an immediate inflammatory response that limits viral replication during the early stages of infection. Important differences between the innate and adaptive immune responses are the relative non-specificity of the innateresponse, the fact that it acts immediately and the lack of memory.

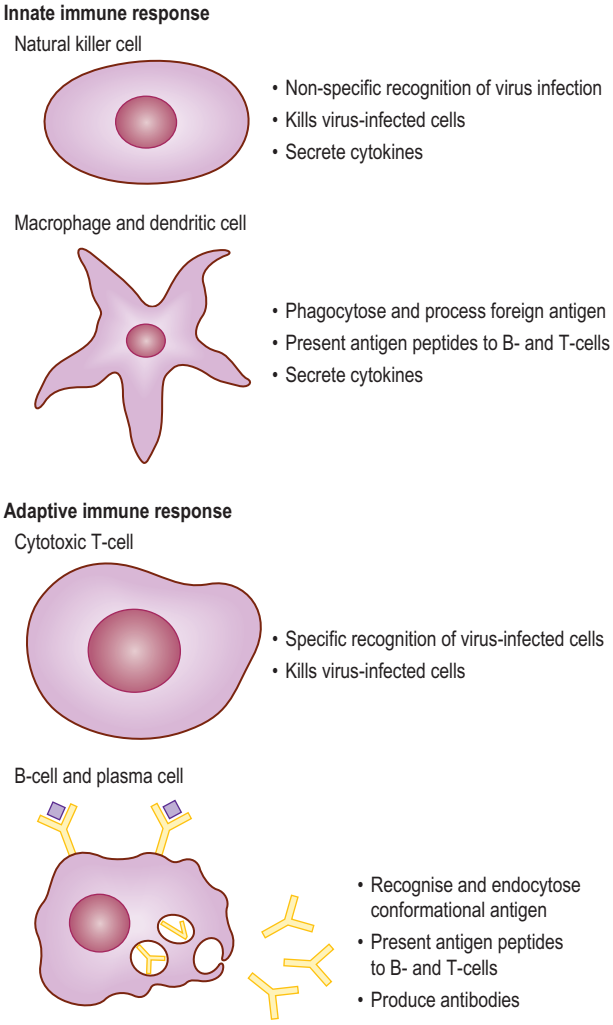


Fig. 1 **Innate and adaptive immune responses.**

The adaptive immune response

The final layer of defence is the adaptive immune response. The adaptive immune response involves two processes: the humoral- and the cell-mediated responses.

The effectors of the humoral response are antibodies; these antibodies are produced by stimulated B-lymphocytes and plasma cells. B-cells contain surface receptors called B-cell receptors, which are similar to the antibodies that the B-cells will produce. These receptors recognise conformational epitopes on foreign agents. B-cells also endocytose these antigens and act as antigen presenting cells to T-helper cells. In the case of virus infection B-cells usually need the help of these T-helper cells, which provide co-stimulation that results in B-cell activation. B-cell activation takes place in the lymph nodes where activated B-cells mature and become antibody producing plasma cells. These cells make different classes of antibodies. During a primary infection IgM is produced first and is later replaced by IgG which is the predominant antibody associated with immunity. IgA is produced by B-lymphocytes of the mucosal associated lymphoid tissue (MALT) and plays a role in protecting these mucosal

- How does the human host compensate for the fact that infectious agents such as viruses have a much shorter regeneration time and could thus evolve much faster than the human host?
- Why is cell-mediated immunity especially important for protection against agents such as viruses that cause intracellular infection?

surfaces. Antibodies have antiviral action by neutralising viruses through binding specific proteins on the surface of viruses, preventing their binding to cell surface receptors. Antibodies may also aid in the killing of virus infected cells by binding antigens on the surface of these cells – these antibody bound cells are recognised by macrophages and natural killer cells which will secrete substances that kill these infected cells. This is called antibody dependent cell-mediated cytotoxicity (ADCC).

The effectors of the cell-mediated response are cytotoxic T-lymphocytes. These cells recognise and destroy virus-infected cells by recognising the peptides associated with major histocompatibility complex I (MHCI) on the surface of infected cells. T-helper cells are also needed for effective activation of these cytoxic cells.

It is thus evident that T-helper cells are important in stimulating both the humoral and cellular immune response. Two types of helper cells are recognised. Th-1 cells direct the immune response towards a cell-mediated response that is usually required in case of viral or other intracellular infections. Th-2 cells direct the immune response towards a humoral and especially anti-parasitic response. A Th-2 response is often ineffective or even detrimental in case of viral infections.

The adaptive immune response differs from the innate response in the fact that it is highly antigen specific. Secondly, it also has a lag time of a few days since antigen-specific immune cells first have to be activated and proliferate. The production of certain cytokines and changing of receptor populations follow B- or T-cell activation which results in proliferation and differentiation of clones of cells that recognise and respond to specific antigens. This proliferation provides more cells that would recognise this antigen and respond – thus enhancing the specific immune response. From this follows the third difference – the adaptive immune response has memory. During differentiation and expansion of activated T and B cells a certain population transforms into long-living memory cells which will become activated and rapidly proliferate should they be stimulated by the same or a similar agent.

Escaping the immune system

Over the ages, viruses have evolved various strategies to escape and survive in the presence of an active immune system. Some viruses mutate rapidly changing their surface proteins to escape antibody pressure. Other viruses hide from the immune system by becoming latent or by infecting immune privileged sites such as the brain. Many viruses encode proteins that interfere with the innate and adaptive immune responses.

Active and passive immunity

Babies are born with a lot of maternal IgG antibodies that were transferred across the placenta during pregnancy. IgA antibodies are also transferred by breastfeeding. Antibody preparations can also be given by injection to prevent or treat certain infections. Since these antibodies were not produced by the immune response of the individual it is referred to as passive immunity. Active immunity is the immune response after natural infection or vaccination (see section on vaccines).

Key points

- The skin, respiratory tract and gastro-intestinal tract contain many non-specific factors that prevent infection.
- The innate immune response is the first response to an invading pathogen. It is relatively non-specific and does not have memory. The adaptive response follows a few days later, it is pathogen specific and due to memory becomes stronger after previous exposure.
- The adaptive response has two components: humoral- or antibody mediated and cell-mediated. T-helper cells play an intricate role in facilitating both humoral and cell-mediated responses.
- Viruses are well adapted to escape the immune response using various mechanisms.

Localised and systemic infections

Introduction

The clinical picture caused by a viral infection depends on various factors, of which the location of the virus is an important one, and can influence treatment and prevention. Some infections are limited to one particular site, usually the site of entry, while other infections spread throughout the body.

Localised infection

In a localised infection, viruses infect the cells at the site of entry, and replicate there, spreading to neighbouring cells, but not into the blood, and do not spread to the rest of the body. Re-inoculation, however, can occur, where virus from one infection site can be transferred to multiple other sites on the body, for instance by scratching a molluscum contagiosum lesion. No viraemia occurs, and few or no antibodies are detected in the blood.

For respiratory and intestinal infections, replication is rapid, and the incubation period is short, as no further spread to other systems is required as part of the pathogenesis of disease. These infections are also controlled fairly rapidly by the immune system. In immunoprivileged sites, such as the skin, viruses such as papillomaviruses may produce a slowly developing clinical picture, as they successfully avoid being eliminated by the immune response.

Clinical implications

A localised infection requires a different approach to a systemic infection when it comes to diagnosis, treatment and vaccination. Often diagnosis will require samples from the actual infection site – stool, and not blood, is required to diagnose rotavirus infection, and serology is unreliable for investigating papillomavirus infection, although with some localised infections, such as herpes simplex, serology may be of use. Treatment usually requires local measures, such as surgical removal, local drug or chemical application, although for severe disease, such as rotavirus, systemic support may be needed, even though the viral infection is not systemic. Vaccines against local infections are more complex. A local papillomavirus vaccine would be impractical, and an intramuscular vaccine would need to provide protective local immunity.

Examples

Examples of localised infection include rhinovirus infection, which replicates locally in the respiratory tract, but not systemically. Papillomaviruses cause localised infection in the epidermal cells. Rotavirus causes local infection in the gastrointestinal tract (Fig. 1), but does not spread systemically. Fig. 2 shows papillomata and molluscum contagiosum – local spread can occur, resulting in multiple lesions.

Systemic infection

In a systemic infection (Fig. 3), the virus usually replicates locally, usually in the mucous membranes, for several days, and then drains into the local lymph nodes via the lymphatic system. There it replicates again, after which it is released into the blood. This is the primary viraemia. From there it travels throughout the body, and replicates in permissive organs and tissues. This allows for a high concentration of virus to be produced, which then enters the blood, causing the secondary viraemia. From there it spreads to further organs, such as the skin in the case of varicella, and it is in this period that the typical clinical picture of the disease usually presents. The secondary

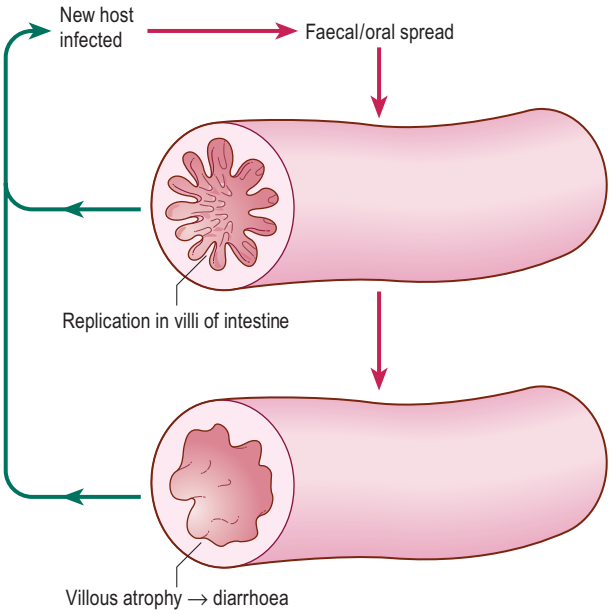


Fig. 1 **Diagrammatic representation of rotavirus infection in the intestines.**

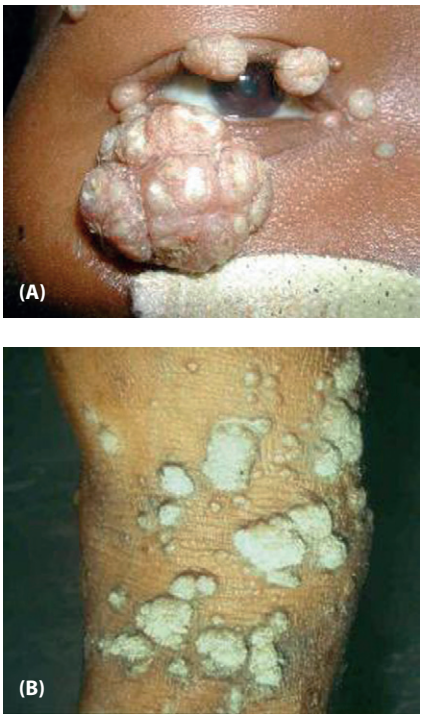


Fig. 2 **A, Molluscum contagiosum. B, Verucca vulgaris.** (Photos courtesy of Prof HF Jordaan, University of Stellenbosch.)

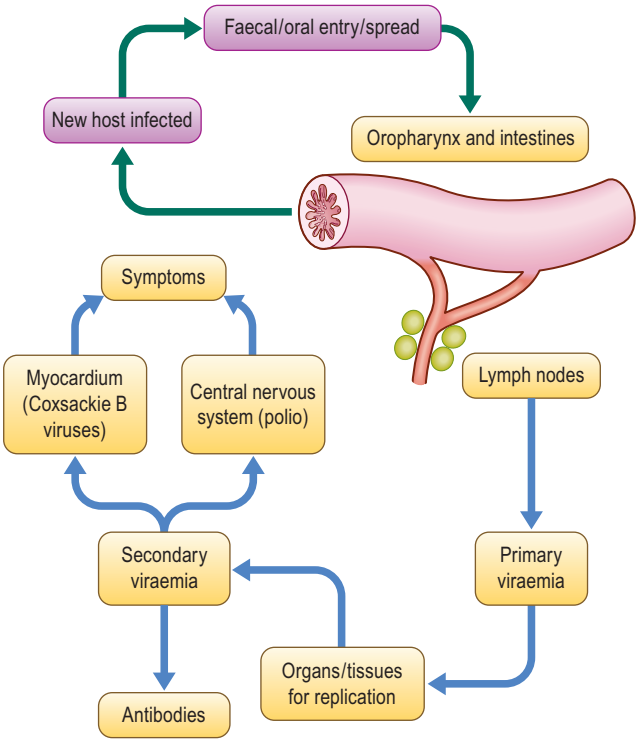
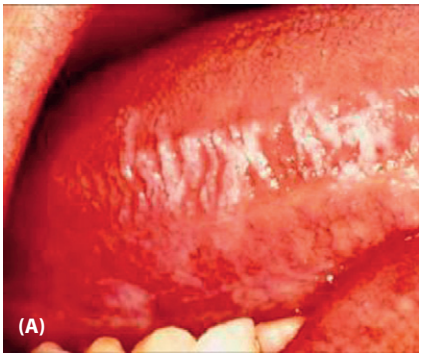


Fig. 3 **Diagrammatic representation of enterovirus causing a systemic infection.**



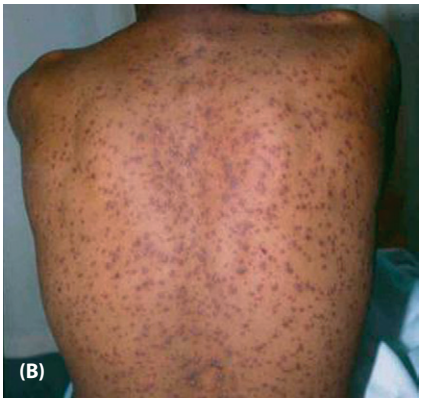


Fig. 5 **A, Oral hairy leukoplakia on the tongue, a manifestation of Epstein-Barr infection, a systemic disease. B, Varicella.** (Photos courtesy of Prof HF Jordaan, University of Stellenbosch.)

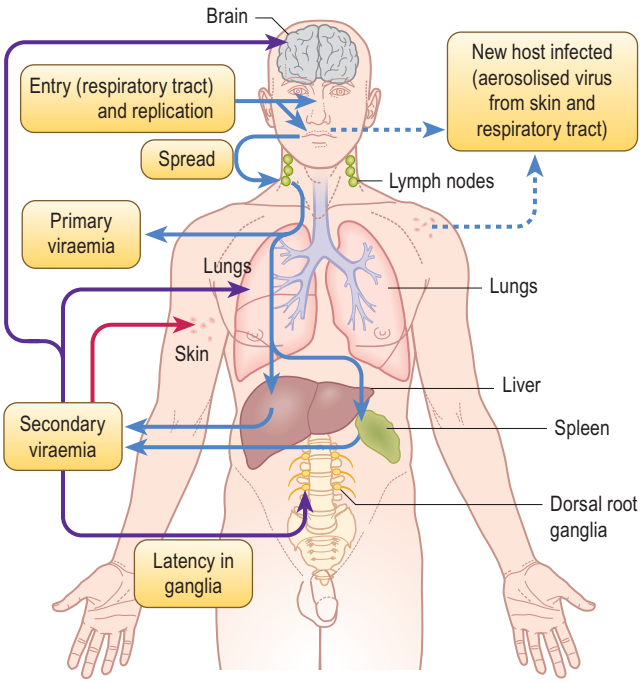


Fig. 4 **Diagrammatic representation of varicella virus causing a systemic infection.**

viraemia elicits a significant immune response, which in turn attempts to control the infection. In the case of mumps and rubella, this is usually effective; in the case of HIV, the immune system does not eliminate the virus from the body. In the case of varicella (Fig. 4), the virus achieves latency, and can reactivate later, usually limited to a local infection spreading via neurons to the skin, although in immunocompromised individuals, systemic infection can be significant.

The period when the virus is still replicating in the local mucosae is also known as the eclipse period, or the viral eclipse.

Clinical implications

Diagnosis of a systemic infection takes the sites of viral replication into account, rather than merely the sites of symptoms. Rubella and cytomegalovirus, for example, are excreted in the urine, even though the urinary tract is asymptomatic. Poliovirus may cause neurological disease, but both cerebrospinal fluid and stools are of value in the diagnosis, as primary replication occurs in the intestinal tract. A more consistent antibody response results from a systemic infection, making serology a more useful tool. Specific treatment, if any, is usually systemic. Preventative measures may be directed at the site of initial replication, such as with the live attenuated polio vaccine, or may be aimed at a systemic immune response, as with the rubella vaccine and the killed polio vaccine.

Examples

Viral infections such as influenza, measles, mumps, rubella and varicella all cause systemic infection. Fig. 5 shows manifestations of Epstein-Barr virus and varicella virus infection.

Acute, chronic and latent infections

This chapter deals with the principles of acute, chronic and latent infections, and may give examples of viruses to demonstrate these principles. It is beyond the scope of this chapter to give details about how each individual viral infection is unique.

Acute primary infection

In an acute viral infection (Fig. 1), the virus replicates without the immune system having any recall for that virus' antigens. The body, therefore, relies on innate immune response initially, and the virus replicates at the point of origin, and, depending on the virus, may spread to local lymph nodes and then to further organs via the blood, for example Varicella. Some viruses, such as human papillomavirus, only replicate locally, although a systemic antibody response may be seen.

Soon after infection, as viral levels are increasing, the immune system responds, triggering a cellular as well as humoral immune response. IgM usually appears first, and then disappears after the acute infection; IgG appears after IgM, and may continue for long periods, sometimes for life. Depending on the site, other immunoglobulins, such as IgA on mucosal surfaces, may be significant in controlling infection and preventing re-infection.

Symptoms usually coincide with the period of viral replication, and usually subside after the virus is cleared. The arrow indicates the possibility of post-infectious symptoms – often due to immune responses, e.g. certain rashes, or Guillain-Barré syndrome. Varicella is shown in Fig. 6 of Chapter 49, as an example.

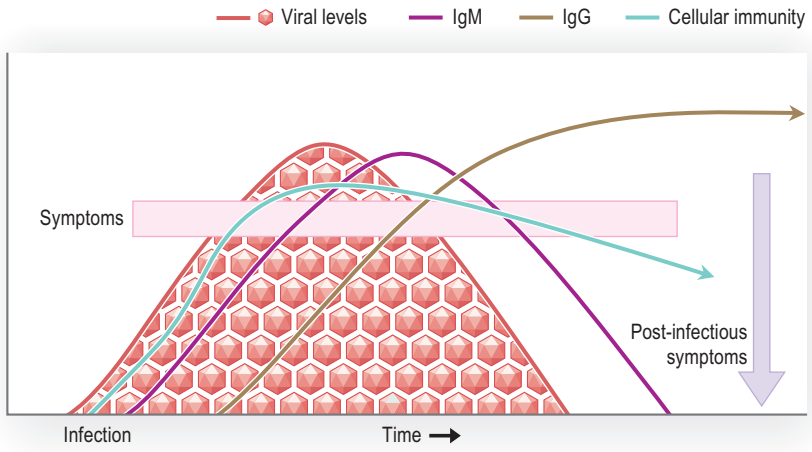


Fig. 1 **Acute infection – primary infection.**

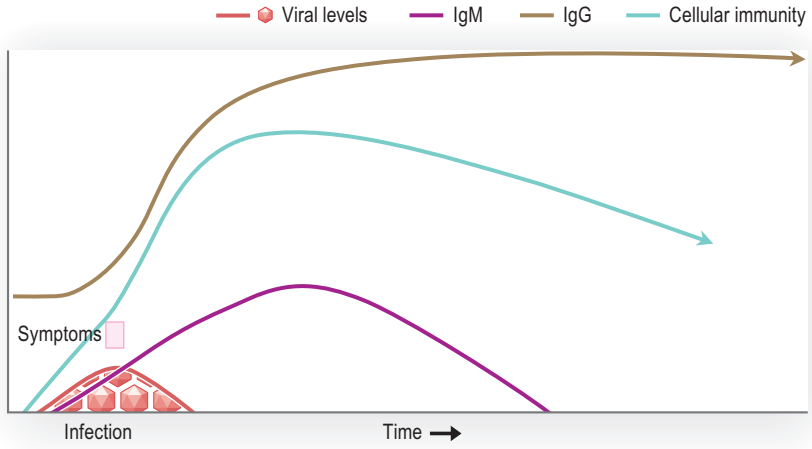


Fig. 2 **Acute infection – secondary infection.**

Acute secondary infection

When a re-infection (Fig. 2), occurs with the same virus, the immune system is able to rapidly respond, as immune memory occurs. Viral replication is suppressed more quickly than with a primary infection, and levels do not usually become significant. Symptoms are often absent and, if they occur, they are usually much milder than with a primary infection.

It should be noted that with some acute infections, such as influenza, one strain may not provide optimal immunity against another strain, and re-infections may present in the way primary infections do. The extent to which the immune response to one virus is protective against a related virus and the extent to which immune memory is able to prevent symptoms with a re-infection both depend on the virus in question.

In some cases, re-infection with a related strain leads to worsening of symptoms, or different symptomatology, such as with Dengue virus infection, where immunity to one strain enables the replication of other strains via uptake into monocytes; the virus therefore replicates by antibody-mediated enhancement of infection. It is in cases such as these that Dengue haemorrhagic fever and Dengue shock syndrome occur.

Chronic infection, reactivation and disease progression

In some cases, clearance of the infecting virus is not possible. Viruses have a variety of methods to escape from the immune system, and some enable the virus to remain in the host indefinitely. Some chronic infections can eventually be cleared, although some are rarely cleared and some cannot be cleared.

These escape methods include:

- integration of the viral genome into the host's genome (e.g. HIV)
- suppression of MHC-I and MHC-II molecules, preventing the immune system from killing infected cells (e.g. herpesviruses)
- up-regulation or down-regulation of cytokines or cytokine response (e.g. HIV), thereby altering immune responses
- inhibition of viral replication to induce latency (herpesviruses)
- infection of immune-privileged areas, where the immune system cannot eliminate the virus (e.g. human papillomavirus)
- infection of lymphocytes
- mutation that changes the viral antigens so that previous immune responses are not effective against the mutant (e.g. HIV)
- blocking apoptosis pathways (e.g. human papillomaviruses).

In cases where viral infection becomes chronic (Fig. 3) viral levels may vary and the degree to which symptoms are experienced may vary. Hepatitis B virus, for instance, may have occasional flare-ups of infection in addition to progressive liver damage, while HIV exhibits a slow disease progression. Some individuals are unable to clear viruses that most people can clear, for instance rare cases of chronic poliovirus excretion or rubella virus in congenitally infected infants.

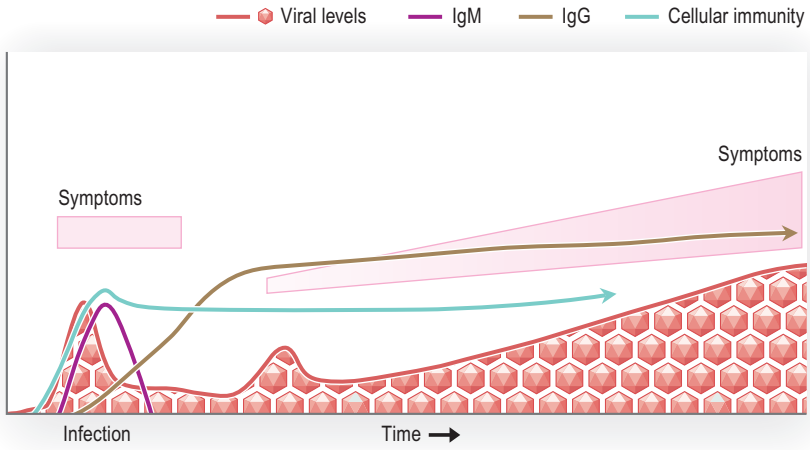


Fig. 3 **Primary infection followed by chronic infection, with periods of reactivation, and disease progression.**

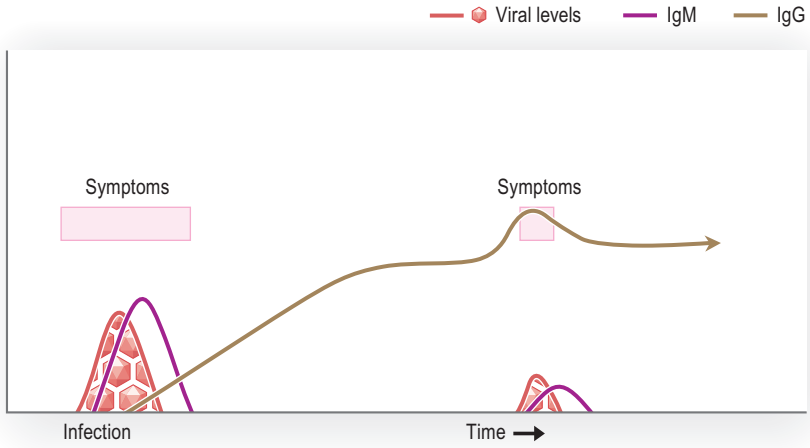


Fig. 4 **Primary infection followed by latent infection, with a period of reactivation.**

Latent infection and reactivation

Chronic infection (Fig. 4) is characterised by some degree of replication during the phases where the virus is not active. Examples of this would be rubella virus in cases of congenital infection, where replication levels are high, and HIV, where levels of virus increase over time.

Latent infection is characterised by absence of replication during inactive periods. Varicella virus, for instance, exhibits true latency, and can reactivate as shingles (Fig. 5). Other viruses that undergo periods of true latency include hepatitis B virus and JC virus. Viruses that can undergo true latency may also replicate at low levels, as in the case of chronic hepatitis B infections.

The progression of disease is similar to that described under chronic viral infections – periods of active replication can occur, with return of symptoms. When such reactivation occurs, IgM levels may again be detectable.

An example of a latent viral infection that causes disease when it reactivates in an immunocompromised host is JC virus, causing progressive multifocal leukoencephalopathy (PML), and also varicella, which causes shingles when it reactivates.

An example of a chronic viral infection that causes disease when it reactivates is chronic hepatitis B infection.

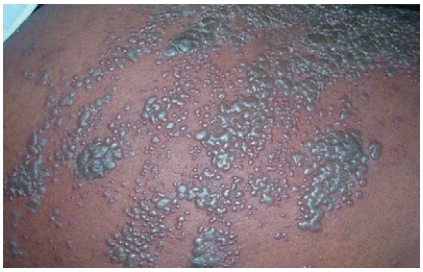


Fig. 5 **Varicella zoster virus reactivates as shingles.** (Photo courtesy of Prof. HF Jordaan, University of Stellenbosch.)

Epidemiology

Definition

Epidemiology is the study of the distribution and spread of disease in human populations. It describes this in terms of person, time and place, and factors associated with and the impact of interventions on disease occurrence.

Patterns of disease occurrence

In order to describe disease occurrence one must differentiate between prevalence and incidence. **Prevalence** refers to the number of individuals who have a condition at **a certain time** whereas **incidence** refers to the number of **new cases** in a particular time period (usually 1 year). **Attack rate** refers to the number of people who are infected who develop **symptomatic disease**.

Seroprevalence refers to the number of individuals who have **antibodies** to a particular agent. In the case of acute infections like measles or hepatitis A virus the seroprevalence refers to people who have been infected or vaccinated and now have antibodies and are thus immune. However, in case of HIV it is indicative of those people currently infected since people do not clear their HIV.

There are different occurrence patterns for viral disease which can either be **sporadic**, **endemic**, **epidemic** or **pandemic**. Sporadic viral infections occur when the risk of infection is low or when very few infected patients are susceptible to disease. An endemic occurrence refers to a constant high incidence of infection which also means that infection is acquired early in life. This is the case for hepatitis A virus, which is faeco-orally transmitted. In rural Africa and India most children are infected before they reach the age of two. Since infection is acquired at an age where hepatitis A virus infection is usually asymptomatic the attack rate, and thus the incidence of symptomatic hepatitis A virus disease, is low in these areas. However, in certain industrialised countries where hygiene has historically improved this was associated with an increase in incidence of symptomatic hepatitis A virus, since the age of acquisition has increased to older children and adults when the attack rate of symptomatic hepatitis A is much higher. This is referred to as the **hygiene paradox**.

During an epidemic there is a sudden increase in the number of infections above the normal occurrence. Certain viruses that have a seasonal pattern of occurrence always cause epidemics. This is the case for most respiratory viruses such as influenza and respiratory syncytial virus. Measles virus has a very high rate of spread, a **reproductive rate** of 12–18, which refers to the number of people infected by each infected individual in a susceptible population. This spread quickly exhausts all susceptible individuals in a closed population and therefore always causes epidemics. The **effective reproductive rate** during and epidemic can, however, be altered by vaccination, which reduces the number of susceptible people. When there are enough people that are immune in a population, the effective reproductive rate will drop below 1 and the outbreak will die out. The ratio of immune people that can prevent or limit the spread of an epidemic is referred to as herd immunity. For measles virus sufficient herd immunity is achieved only when more than 95% of the population is immune, but for a less infectious virus such as polio or rubella a percentage of 80–85% is required. When the herd immunity drops due to the increase in non-immune individuals (either by birth, immigration or loss of immunity) to such an extent that an epidemic can again occur, the population has reached an epidemic threshold.

Patterns of spread

Epidemics or outbreaks occur when individuals are infected from a common source (such as a contaminated food or water supply or an infected blood product) or when the infection can be transmitted from person to person either directly or by vector borne transmission (see section on transmission patterns). Fig. 1 illustrates the difference between a common source outbreak, when the number of new cases over time shows a unimodal distribution with a sharp peak, versus an epidemic with limited secondary spread, where there can be more than one peak or an expanding epidemic with human-to-human spread and a continuous rise in the number of cases.

Viral reservoir

Viruses that are maintained by animals with intermittent infection of humans are said to have an animal reservoir. These infections are referred to as zoonoses. This is true for viruses like rabies and some of the arboviruses (viruses transmitted by arthropod vectors between hosts, either human, bird or animal).

Humans chronically infected also act as reservoir – this is true for conditions like HIV and hepatitis B and C. Humans also act as a reservoir for viruses that become latent with intermittent reactivation such as herpes simplex virus.

Virgin-soil epidemics

These are epidemics that occur in populations not previously exposed to an infectious agent, either through natural infection or immunisation. Common childhood illnesses such as measles have a low mortality when healthy children are infected. However, should a population of non-immune adults be exposed to a virus towards which there is no underlying immunity, the mortality rate may be very high. Such was the case in 1875 when nearly one-third of the people of Fiji died as measles was first introduced. Similar epidemics occurred with measles in Iceland and the Faroe Islands with the re-introduction of measles after years of absence. Virgin-soil epidemics also led to severe mortality with the introduction of smallpox in the Americas. The pandemic influenza virus of the 1918–1919 pandemic and the recent SARS outbreak are other examples where the introduction of new viruses was associated with excessive mortality.

STOP THINK

- Why can the recent SARS outbreak and a future influenza pandemic be seen as virgin-soil epidemics?
- Why is the mortality increased in a virgin-soil epidemic?
- Why are mass immunisation strategies in addition to routine infant immunisation often needed to stem an outbreak?

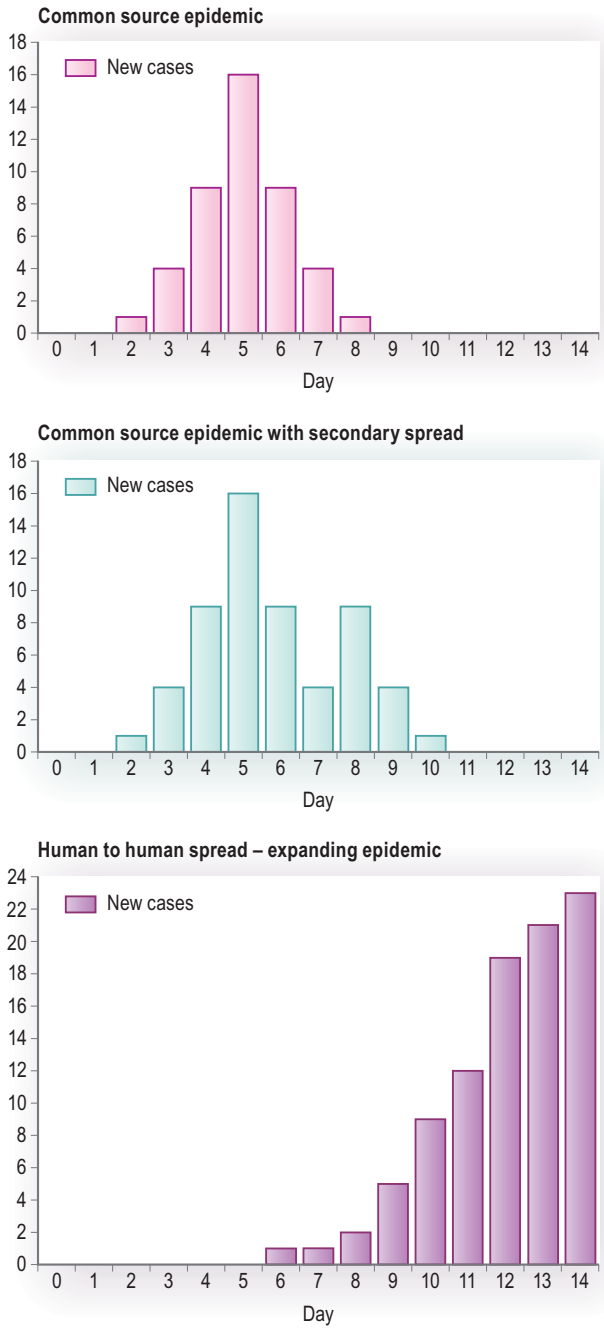


Fig. 1 **Outbreak patterns.**

Tools of the trade: epidemiological studies

Different methods are available to access the prevalence and incidence of viral disease and determine the risk factors associated with infection and disease.

Cross sectional studies such as seroprevalence measure the ratio of individuals with antibodies towards a specific agent in the population. An example of such a study is antenatal clinic HIV seroprevalence studies which are used in developing countries as a measure of the percentage of HIV infected patients in the population. Measuring incidence for acute diseases relies on disease surveillance, which depends on accurate notification of new cases. For chronic disease this is more difficult, but an estimate can be made using age-prevalence differences or special techniques to detect new infections.

Case-control studies are efficient studies to determine risk factors such as common exposures and their effect on the likelihood of being infected. Cohort studies which follow a population over time to detect different risk factors of disease are more expensive and time consuming, but can provide unforeseen associations with disease occurrence. Molecular epidemiology has become a very useful tool in the investigation of outbreaks: Viruses infecting cases during the outbreak are sequenced and these sequences are compared to each other and to other current or historic outbreaks. This enables one to study the source of outbreaks or transmission chains and provides valuable data for infection control and prevention.

Key points

- Endemic circulation occurs when there is a high rate of infection, early in life, in a population.
- An epidemic is a sudden increase in the number of cases above what is normally expected.
- The effective reproductive rate refers to the number of individuals infected by each infectious individual. The ratio of immune people will thus impact on the effective reproductive rate.
- Epidemics occur either when viruses are acquired from a common source or when it is transmitted from person to person (directly or vector borne).

Emerging and re-emerging viral infections

Not so long ago, many experts believed that infectious diseases were a thing of the past. Sir Frank Macfarlane Burnet (1899–1985), famous Australian virologist and immunologist and recipient of the 1960 Nobel Prize in Physiology or Medicine, said in 1962: '… one can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious disease as a significant factor in social life.' William Steward, Surgeon General of the USA, stated before the US Congress in 1969: 'The time has come to close the book on infectious diseases.' Looking at how markedly deaths due to infectious diseases in the industrialised countries had declined during the course of the 20th century, one understands why these statements seemed reasonable at the time.

However, they were wrong. Not only did most developing countries fail to experience the same improvements, but also the industrialised world saw a resurgence of infectious diseases, beginning with the advent of the AIDS pandemic in the 1980s.

To understand why viruses (and other infectious agents) may emerge (or re-emerge), one needs to analyse the interplay between the natural world and humanity. The natural world comprises an immense number of different species and is thus an inexhaustible source of novel infectious agents. Their 'spill-over' into the human sphere, which includes domestic animals, can occur due to numerous natural and man-made factors, as illustrated in Fig. 1 depicting the 'host-parasite ecological continuum'. Humanity has since pre-historical times been subject to the introduction of infectious diseases (Fig. 2). It is believed that measles, for example, originated when cattle were domesticated. The ensuing constant close contact between cattle and human beings allowed the cattle-specific morbillivirus, Rinderpest virus, to evolve into the closely related human-specific morbillivirus, measles virus, until today a major cause of childhood mortality. The human immunodeficiency virus (HIV) emerged in a similar way: originally a zoonosis (infection transmitted from animals to human beings), it is now a pathogen that is transmitted exclusively from person to person. Other viruses continue to affect human beings through zoonotic transmission, i.e. from their animal reservoir, but there are huge differences in their ability to establish ongoing subsequent transmission from person to person.

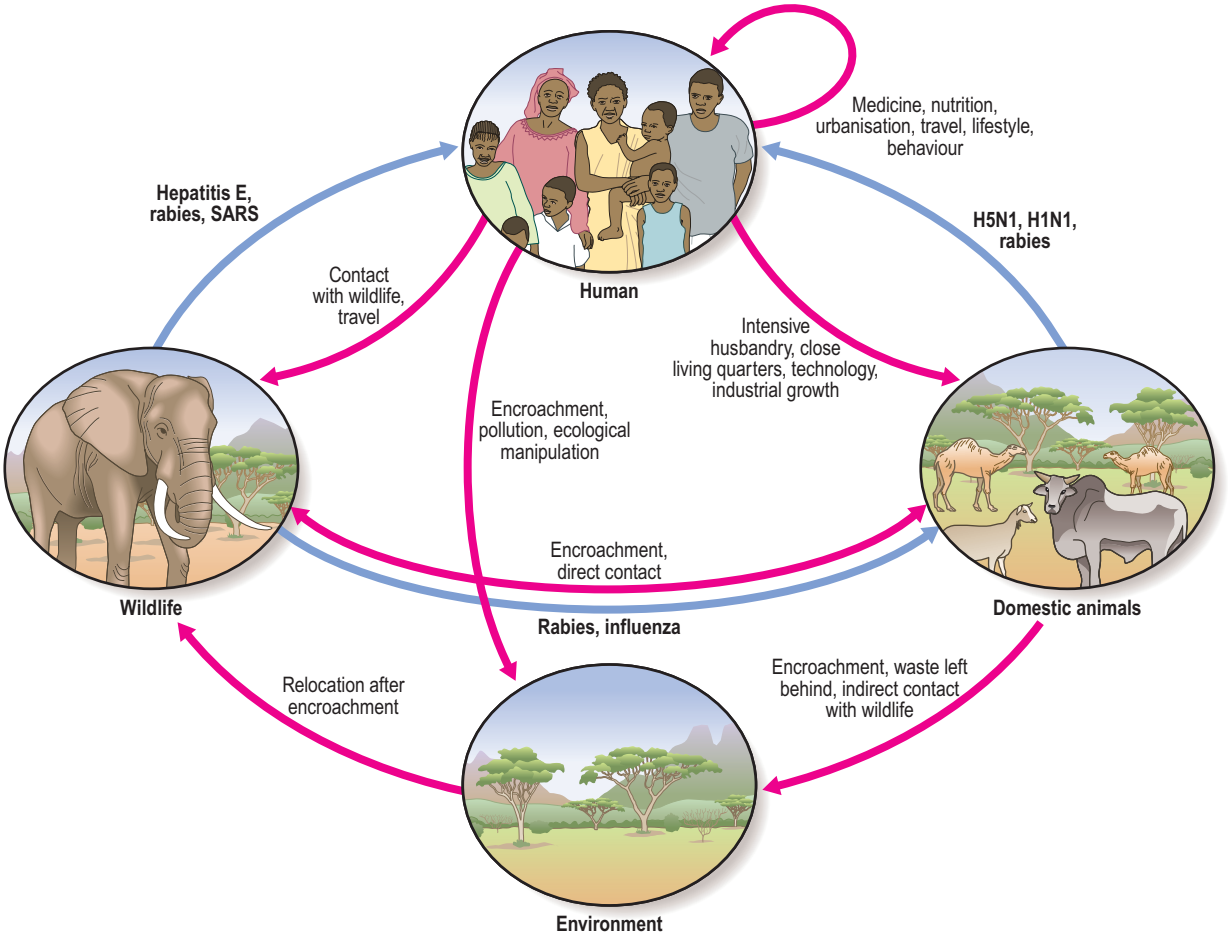


Fig. 1 **Interaction between animals, humans, and the environment resulting in emergence of new infectious diseases.** (Adapted from Daszak *et al.*, 2000.)

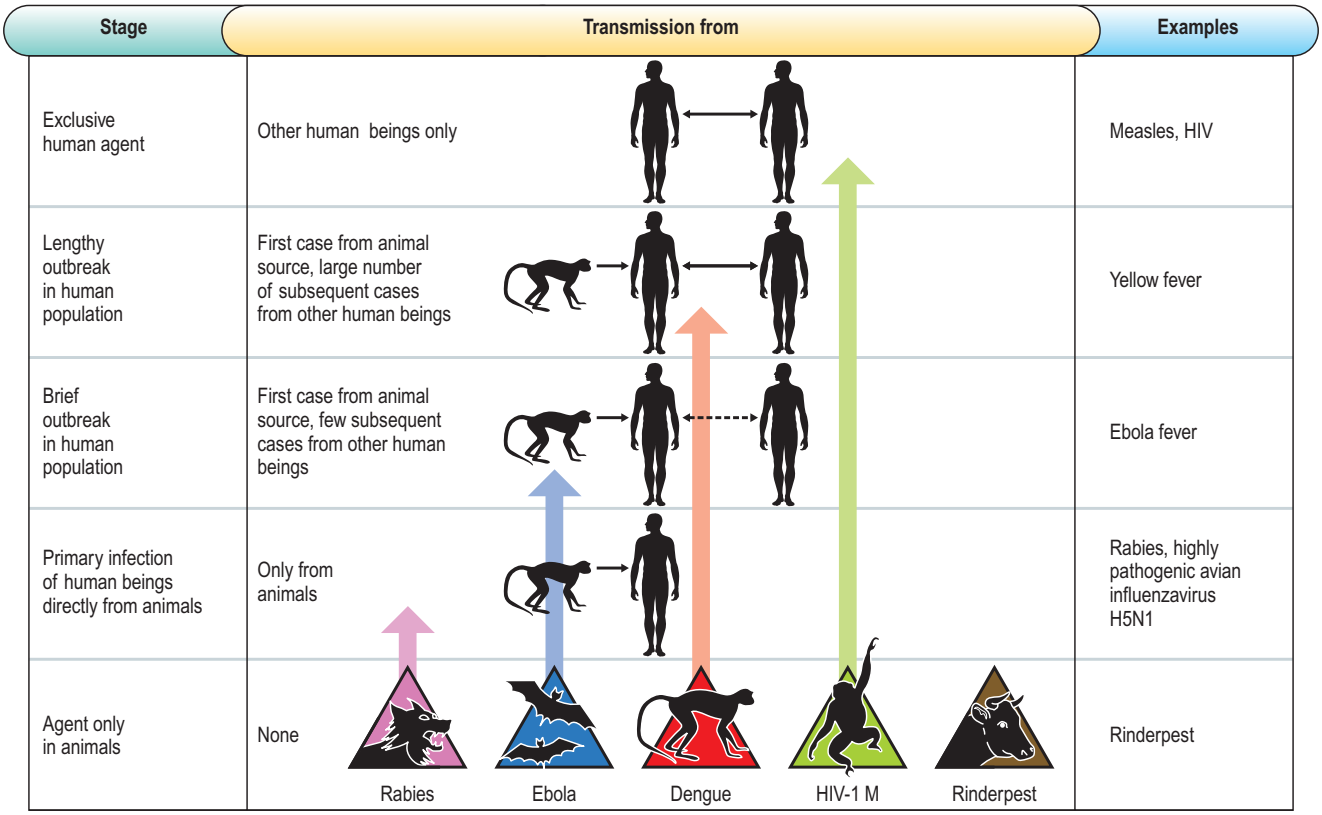


Fig. 2 **Stages of the emergence of new infectious diseases.** (Adapted from Wolfe *et al.*, 2007.)

Box 1 **What 're-emerging' means**

The term (re-)emerging viral diseases comprises different entities:

1. New viruses appearing for the first time in human beings. These novel viruses are mostly of animal origin (i.e. zoonotic). Examples are HIV, Nipah virus, SARS and new influenzavirus types.
2. Known viruses with increasing relevance, rising incidence, or expanding geographical distribution. This 're-emergence' may result from new (medical) technologies and procedures (e.g. transfusion and transplantation: cytomegalovirus, hepatitis C virus), or from faltering control efforts (e.g. vector-borne viruses: dengue, yellow fever) etc.
3. Newly recognised agents of previously known diseases should more aptly be called 'emerging diagnosis' and are not truly emerging. Examples include hepatitis E virus, Kaposi's sarcoma-associated herpesvirus and others.

Box 2 **Factors leading to the emergence of new infectious diseases**

The following factors may contribute to the emergence of novel infectious disease agents (from Smolinski *et al.*, 2003: Institute of Medicine report):

- Microbial adaptation and change
- Human vulnerability
- Climate and weather
- Changing ecosystems
- Economic development and land use
- Human demographics and behaviour
- Technology and industry
- International travel and commerce
- Breakdown of public health measures
- Poverty and social inequality
- War and famine
- Lack of political will
- Intent to harm

Further reading

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The laboratory diagnosis of viral infections – introduction and principles

Diagnostic virology is still a relatively young field, having only gained importance in the late 1940s. However, since then detection techniques in virology have evolved dramatically. These methods can be thought of simply as two types: those which detect the virus or parts of the virus (direct) and those which detect the body's response to a viral infection (indirect) (Box 1).

Box 1 **Principles of viral diagnosis**

Direct diagnosis: through detection of the infecting virus itself

- Visualization of virus particles: electron microscopy
- *In vitro* propagation: virus isolation on cell culture, embryonated eggs or in experimental animals
- Visualization of the effects of viral infection on infected tissue or cell cultures: light microscopy
- Detection of viral antigens: immunostaining of infected cells or tissues; detection of viral antigen in body fluids or excretions
- Detection of viral nucleic acid (viral genome): qualitative ('yes or no' answer); quantitative ('how much?'); genotyping ('what type?')

Indirect diagnosis: through detection of the infected host's immune response

- Humoral immunity: antibodies of different classes (IgG, IgM, IgA) and subclasses and of different avidity
- Cellular immunity: cytotoxic T-cells

Electron microscopy

Electron microscopy is the only technique that allows direct visualisation of the virus (Figs. 1 and 2). Identification is based on the typical morphological features of the virus. These include the size, shape and ultrastructural features.

This is a catch-all approach, but it has limited sensitivity, requiring about 10^6 viral particles/ml of fluid to allow accurate visualisation and identification. Electron microscopy cannot differentiate between viruses of the same family, e.g. herpes simplex virus (HSV) cannot be differentiated from VZV, unless further tests are done on the sample, e.g. solid phase immune electronmicroscopy (SPIEM).

Cell culture/virus isolation

In vitro propagation of viruses may utilise embryonated eggs or experimental animals; today cell culture is the preferred medium for most purposes. Virus isolation has been relegated to a few centralised reference laboratories.

Many different types of cell culture exist, as no particular cell line supports the growth of all important viruses (Table 1). Different cell types can be classified into three groups: primary cell lines, finite, continuous.

Primary cell lines are prepared directly from tissue, usually an animal or embryo. Primary cells can be passaged (i.e. transferring some cells to a new container and allowing the cells to multiply) only for a few generations before dying.

Secondary or finite cell lines are also known as diploid and can be passaged for about 40–60 passages.

Continuous cell lines are transformed cell lines that are 'immortalised' and can be passaged indefinitely. Most cultures are incubated at 35–37°C, although lower temperatures (33°C) are used for some respiratory the presence of virus in cell culture viruses.

The main method for diagnosing the presence of virus in cell culture is by detecting changes in the cell structure by examination with a light microscope known as cytopathic effect (CPE) (Fig. 3a and b).

Haemadsorption is a method used to reduce the time taken to detect a virus growing in culture. Glycoprotein structures, called haemagglutinins present on the surface of some viruses, e.g. influenza and parainfluenza cause erythrocytes to adhere to cells. The presence of haemadsorbing virus can be detected days before CPE.

Some viruses, e.g. rubella, grow without producing CPE. For some of these viruses use is made of the phenomenon known as 'interference'. For example, when rubella is grown in primary monkey kidney cells, these cells become resistant to infection with echovirus. Once a culture has grown an interfering virus such as rubella,

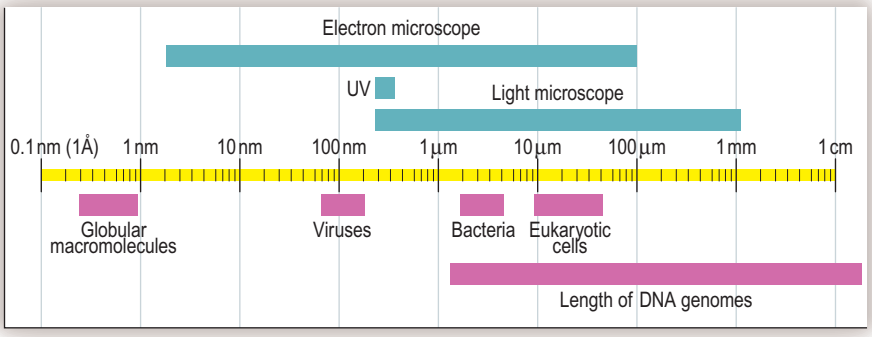


Fig. 1 **Relative sizes of cells, bacteria and viruses.**

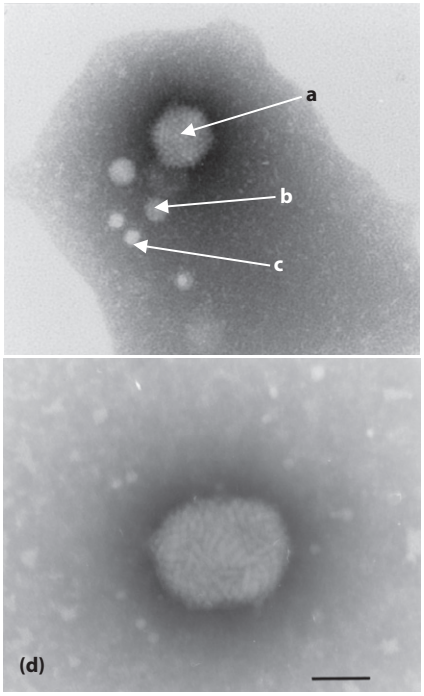


Fig. 2 **Electron microscope photos of a, adenovirus; b, astrovirus; c, parvovirus and d, a pox virus.** (Photos courtesy of Prof M Taylor, Pretoria University.)

neutralisation or staining with a fluorescent antibody can be used to identify the virus.

Shell vial culture combines culture with antigen detection methods and is a means of rapid viral detection. Samples are inoculated onto fibroblasts grown on a coverslip. After incubation immunofluorescent staining is performed using monoclonal antibody.

Virus culture still has a crucial role to play in determining resistance of viruses to antiviral agents, so called phenotypic resistance. This is particularly useful where the genotypic resistance patterns are not well understood, e.g. cytomegalovirus (CMV). Historically viruses were propagated in laboratory animals and embryonated egg. Histopathologists may use a light microscope or even electron microscope to visualise the effects of viral infection on the morphology of the tissue. Immunostaining of cells or tissue using labelled monoclonal antibodies to detect viral antigens in diseased tissue.

Detection of viral antigen

Methods for the detection of viral antigens allow the rapid identification of a wide variety of viruses. These methods employ reactions between virus and labelled antibody.

Table 1 **Advantages and disadvantages of cell culture**

Advantages	Disadvantages
Detects viable virus	Labour intensive
'Catch all'	Not able to culture a number of viruses
Isolate can be studied further	Skilled staff needed
Dual/mixed infections detected	Multiple cell lines required
Inexpensive	Requires large space

Immunofluorescence

Immunofluorescence (IF) a method often used for the detection of viral antigens. It may be used directly on samples, e.g. nasopharyngeal aspirate, on cell cultures or biopsy samples. The advantage of this technique is that it is quick and easy to perform and results can be available in a short period of time. However, it requires a skilled operator and a flourescent microscope. It is a difficult procedure to scale up and non-specific binding can make interpretation of results difficult. Fig. 3c shows CMV detected by IF.

There are two types of IF assay; see Fig. 4.

Table 2 **Frequently used cell lines, with examples of viruses grown in them**

Cell line type	Example of cell line	Example of virus grown
Primary	Monkey kidney	Influenza Parainfluenza Enteroviruses
Finite	Fibroblast	Cytomegalovirus Varicellar zoster virus Adenovirus
Continuous	Hep-2	Respiratory syncytial virus Herpes simplex virus
	Madin-Darby Canine Kidney (MDCK)	Influenza

Key points

- Viral culture is a 'catch-all' method, although is now less used in routine virology laboratories.
- Electron microscopy allows direct visualisation of the virus, but requires high viral titres to be detected.
- Immunoflourescence is useful for detecting both antigen and antibody.

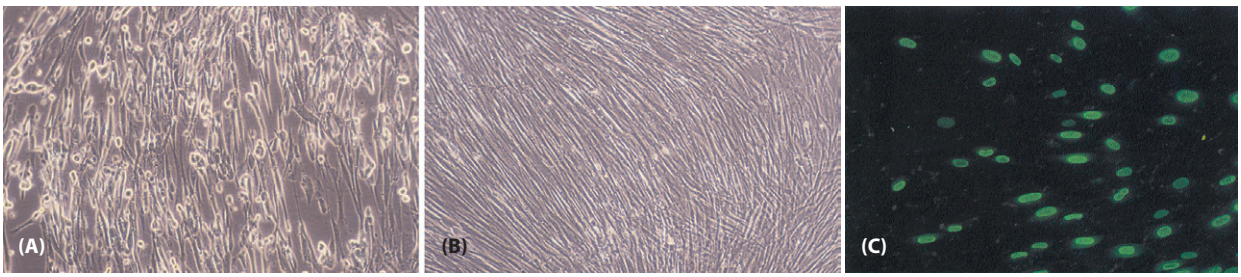


Fig. 3 **Cell culture of CMV grown in fibroblasts. A, Cytopathic effect (CPE). B, Cell culture monolayer of uninfected fibroblasts. C, DEAFF test – Immunofluorescent staining of fibroblasts infected with cytomegalovirus.**

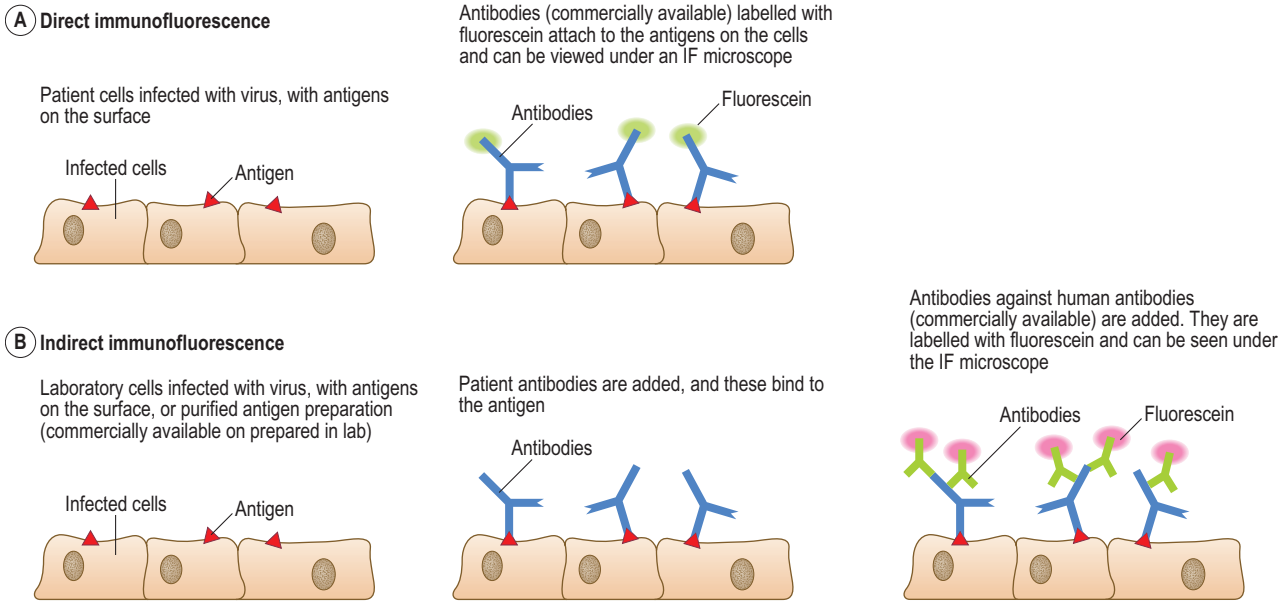


Fig. 4 **Diagrams indicating (A) direct immunofluorescence and (B) indirect immunofluorescence.**

The laboratory diagnosis of viral infections – detection of virus-specific immunity

Antibody testing (viral serology) to diagnose an acute (fresh) infection:

1. Seroconversion or rise in antibody titre:
   - Test 'paired' sera (acute and convalescent sample, 10–14 days apart) in parallel
   - Significant if four-fold rise in titre
   - Problem: retrospective diagnosis!
2. IgM antibody testing:
   - Obtain sample during acute phase
   - IgM appears early, undetectable within months
   - IgM does not cross placenta or blood–brain barrier (useful to diagnose intrauterine or perinatal infections)
   - Problem: often low specificity.
3. Avidity testing:
   - Obtain sample during early (post-acute) phase
   - Antibody avidity increases over time; low avidity antibody suggests a recent primary infection, high avidity antibody an infection or immunisation in the distant past. This is particularly useful in pregnant women suspected of having experienced rubella or another relevant viral infection during pregnancy (Fig. 1).

IgG antibodies are formed later than IgM antibodies and often persist for life. Testing for IgG antibodies can help:

1. to determine immune status:
   - marker of immunity following wild virus infection or active immunisation, for example:
     - to detect anti-HBs level after hepatitis B virus (HBV) immunisation
     - to establish pre-existing varicella zoster virus (VZV) immunity after contact with a chickenpox patient
     - to check for hepatitis A virus (HAV) immunity before travelling.
2. to diagnose persistent (latent or chronic) infection:
   - marker of infection, for example:
     - to determine HIV infection status
     - to assess risk of reactivation of herpesvirus infection in immunosuppressed organ transplant recipients?

Methods to test for virus-specific antibodies

To detect the presence of a specific antibody in a patient's serum, one uses its ability to bind to the antigen against which it is directed. The antigen will be used as part of the antibody test, together with a system that allows the visualisation of the antibody–antigen reaction if it takes place. Classical methods are the haemagglutination inhibition (HAI) and the complement fixation test (CFT). Here, red blood cells are used to make the reaction visible. These tests have been largely replaced by the enzyme-linked immunosorbent assay (EIA or ELISA).

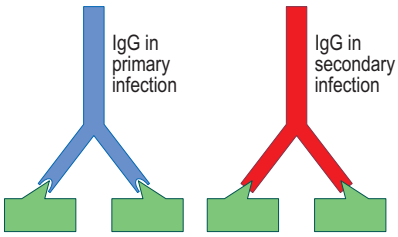
The traditional ELISA is conducted using a 96-well microtitre plate (see Fig. 2). The bottom of each well serves as the solid phase, and the subsequent reactions take place within the well. Between each step thorough washing ensures removal of all unbound reagents before the next reagent is added. The endpoint is the presence of an enzyme coupled to either an antibody or an antigen which is able to change a substrate into a molecule of a different colour. The intensity of the resulting colour change (optical density, O.D.) may be used to measure the activity (amount and functionality) of the antibody that is sought, resulting in quantitative values (often expressed as IU/ml) to determine immunity (Fig. 3). Today, modified semi- or fully automated systems look very different but still use the same underlying principles.

Depending on the assay's purpose, an ELISA may be designed in one of several different 'formats', each with its own strengths and weaknesses (Fig. 4). The antibody ELISA can be used to distinguish between IgG and IgM antibodies, depending on the test antibody employed. The IgM assay may, however, give false-positive results in the presence of rheumatoid factor (an IgM autoantibody reactive against the body's own IgG); if the patient's serum contains specific IgG antibodies that bind to the antigen on the solid phase, rheumatoid factor will bind to these and subsequently be mistaken for virus-specific IgM.

The main strength of the competitive ELISA is its specificity; this is the only format where the intensity of the resulting colour is inversely proportional to the antibody activity. The reverse capture format is particularly useful to test for antibodies in body fluids with low levels of antibodies, such as oral fluid. The advantage of the immunometric or 'sandwich' ELISA is its ability to detect specific antibodies of all classes (IgG, IgM, etc.).

For some purposes, such as screening for HIV infection, rapid

In a primary infection, the antibodies do not bind to the antigen as well as in a secondary infection



A reading is taken of the same sera, one well treated with urea to dislodge the antibodies, and one well untreated

Example of a primary infection

Treated well: OD = 0,259
Untreated well: OD = 1,451

$$\frac{0{,}259}{1{,}451} = 18\%$$

18% < 30% → primary infection

Example of a secondary infection

Treated well: OD = 1,210
Untreated well: OD = 1,482

$$\frac{1.210}{1{,}482} = 82\%$$

82% > 50% → secondary infection

(OD = optical density, read by a machine)

Fig. 1 **Principles of avidity testing.**

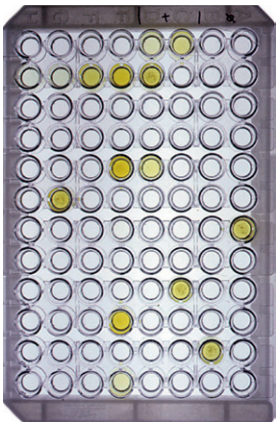


Fig. 2 **An ELISA plate showing positives (yellow) and negatives (clear).**

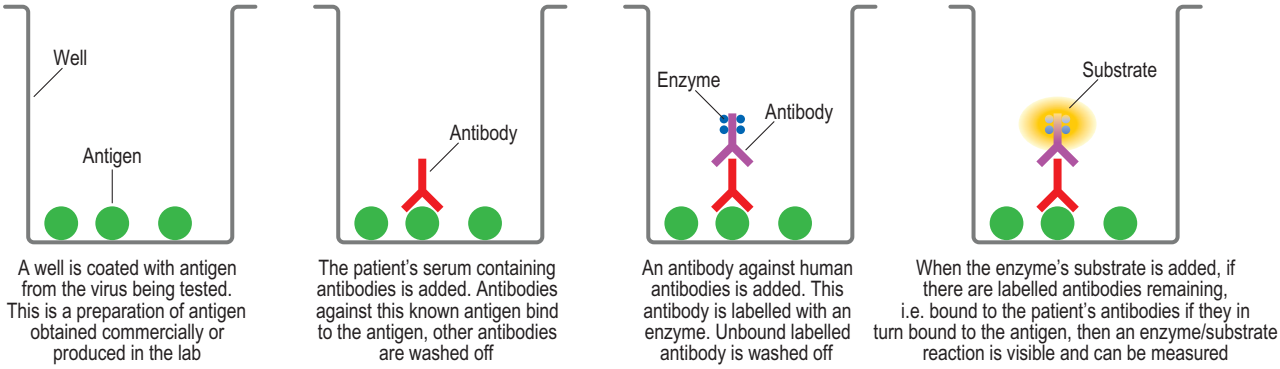


Fig. 3 **Principles of an ELISA.**

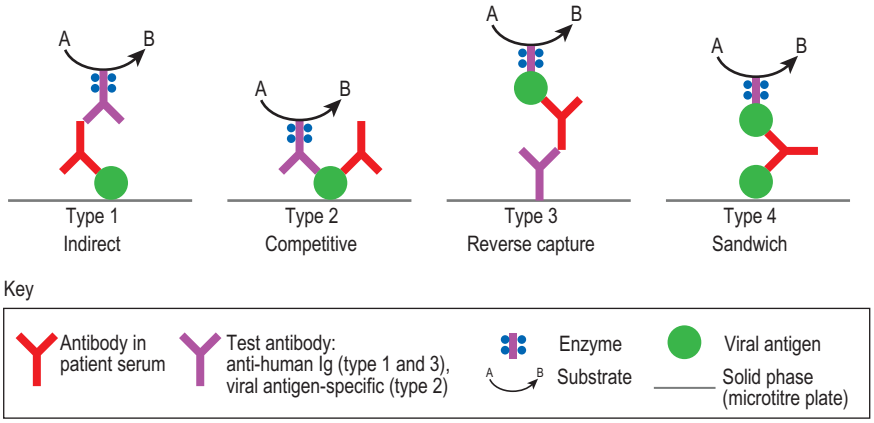


Fig. 4 **Different ELISA formats.**

(also called point-of-care or near-patient) test devices may be preferable to a laboratory-based assay (Fig. 5). The availability of the test result within half an hour or less encourages test uptake and reduces the rate of results that are not picked up by the patients. Seeing how the test is done also tends to increase confidence in the test result and the risk of logistic and clerical errors is reduced. However, such tests are performed by people who are not professionally trained laboratory workers, and quality assurance and control are difficult.

For rapid, as well as laboratory-based, HIV screening tests, the basic principle is that all reactive ('positive') screening test results must be confirmed by at least one more, different test and, ideally, also on a second, independently obtained sample.

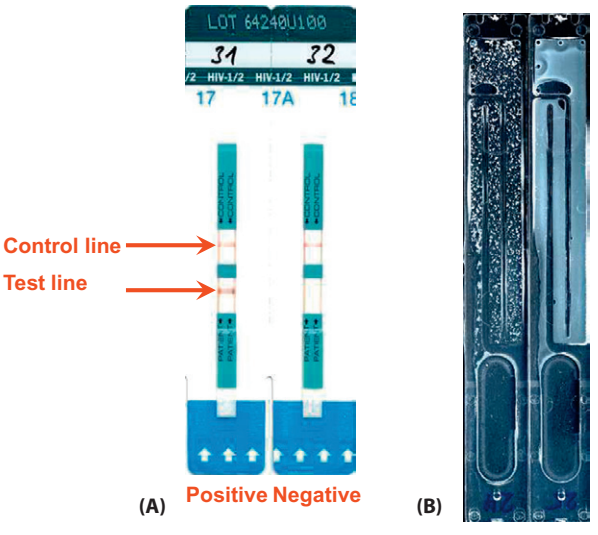


Fig. 5 **Two different HIV rapid tests (A) lateral flow immunochromatography and (B) latex agglutination.**

Box 14.1 **Interpreting test results**

- Sensitivity:
  - Probability that the result is positive if the patient is infected
- Specificity:
  - Probability that the result is negative if the patient is not infected
- Positive predictive value:
  - Probability that the patient is infected if the result is positive
- Negative predictive value:
  - Probability that the patient is not infected if the result is negative

Box 14.2 **Measuring text performance**

		Test result	
		Positive	Negative
real status (reference test result)	positive	true pos.	false neg.
	negative	false pos.	true neg.

$$\text{sensitivity} = \frac{\text{true pos.}}{(\text{true pos.} + \text{false neg.})}$$

$$\text{sensitivity} = \frac{\text{true neg.}}{(\text{true neg.} + \text{false pos.})}$$

} test-dependent

$$\text{positive predictive value (PPV)} = \frac{\text{true pos.}}{(\text{true pos.} + \text{false pos.})}$$

$$\text{negative predictive value (NPV)} = \frac{\text{true neg.}}{(\text{true neg.} + \text{false neg.})}$$

} depend on test and on population

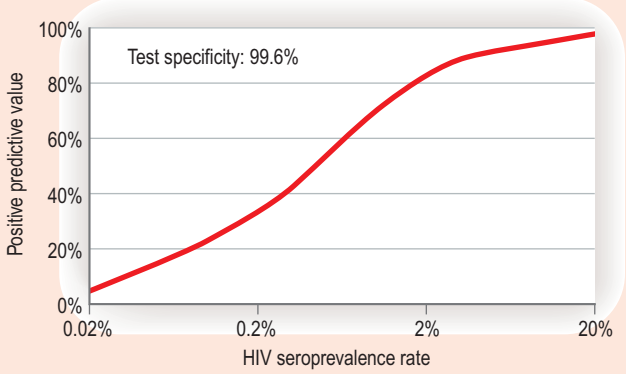


Fig. 6 **Seroprevalence rates influence test performance!**

The laboratory diagnosis of viral infections – detection of viral nucleic acid

The detection of viral genome, i.e. nucleic acid, is a powerful tool. In some viral cases, such as chronic hepatitis B with high replication activity, acute parvovirus B19 infection and acute rotavirus diarrhoea, there is a high virus titre and thus sufficient viral genome in clinical samples for it to be detected and characterized without this tool. The real breakthrough, however, only came with the advent of nucleic acid amplification techniques, which allow for the selective 'enrichment' of target nucleic acid before it is detected.

The best-known of such methods is the polymerase chain reaction (PCR) (Fig. 1). As the name suggests, it is a cyclic reaction going through 30–40 cycles consisting of denaturation (melting) of double stranded DNA at 90–95°C, annealing of primers at 50–60°C, and elongation of primers at 70–72°C, followed by denaturation of the newly formed double-stranded DNA molecule and starting the sequence over. If the viral nucleic acid to be detected is RNA, the reaction is preceded by reverse transcription (RT-PCR).

Its prerequisite is the use of a thermostable polymerase enzyme that can withstand the high temperatures used. It also requires the knowledge of the target molecule's base sequence as specific primers are used that are complementary to portions of the target. In the end, the target sequence (product) has been amplified enormously (ideally by $2^{\text{number of cycles}}$) and is identified either by size through electrophoresis on an ethidium bromide-containing agarose gel and visualization under UV light (Fig. 2) or, more specifically, using labelled probes. This is done in the form of real-time PCR whereby amplification and (quantitative) detection by means of specific probe take place simultaneously (Fig. 3).

There are a number of similar methods for the selective amplification of target sequences, such as ligase chain reaction (LCR), nucleic acid sequence-based amplification (NASBA), branched DNA signal amplification (bDNA) and hybrid capture.

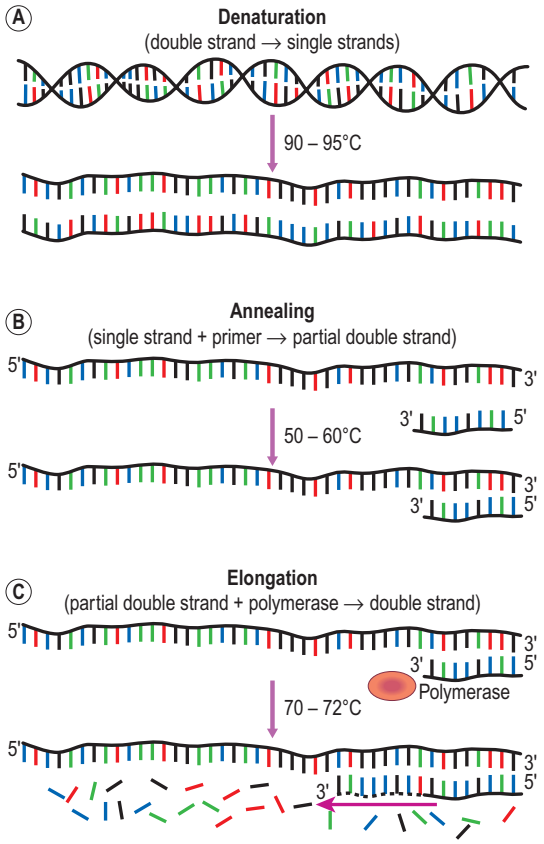


Fig. 1 **The principle of the polymerase chain reaction.**

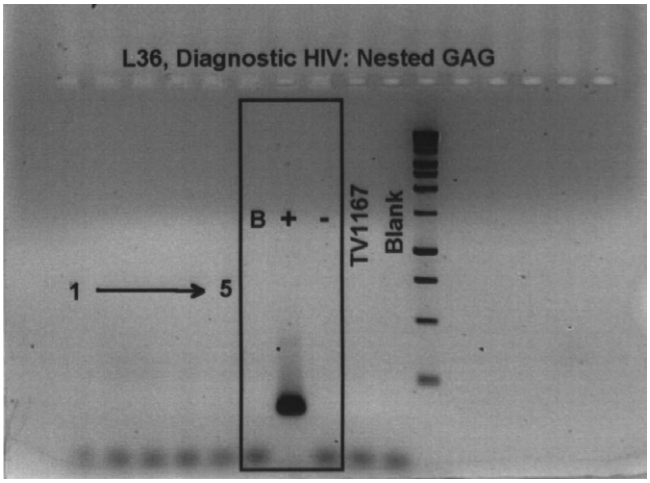


Fig. 2 **Agarose gel electrophoresis, showing five samples (1 → 5), reagent blank (B), positive control (+), negative control (–), and a DNA molecular weight marker.**

So for what purposes is nucleic acid testing (NAT) being used routinely?

In its qualitative form to provide a 'yes or no' answer, i.e. the detection of viral genome serves as a marker of infection: Does the patient have infection or not? NAT is valuable for this purpose if:

- the virus in question cannot (readily) be isolated or otherwise detected, e.g. hepatitis B virus (HBV), human papilloma virus (HPV) or human immunodeficiency viruses (HIV)
- there is a small sample volume or a low infectious dose, e.g. amniotic fluid, cerebrospinal fluid (CSF) to be tested for rubella or herpes simplex viruses
- antibody testing fails, e.g. acute HIV infection ('diagnostic window'); immunosuppressed patients who do not form antibodies well; passively acquired antibodies masking the true infectious status, like in babies born to HIV-infected mothers
- one needs to exclude infectivity, e.g. blood products.

Quantitative NAT ('how much nucleic acid?') is a very important tool particularly to guide optimal antiviral treatment. It can serve:

- as a therapeutic marker to monitor antiviral chemotherapy, e.g. HIV viral load testing of patients on antiretroviral therapy to ensure 'full suppression'
- as a prognostic marker, e.g. HIV viral load predicts survival in AIDS patients

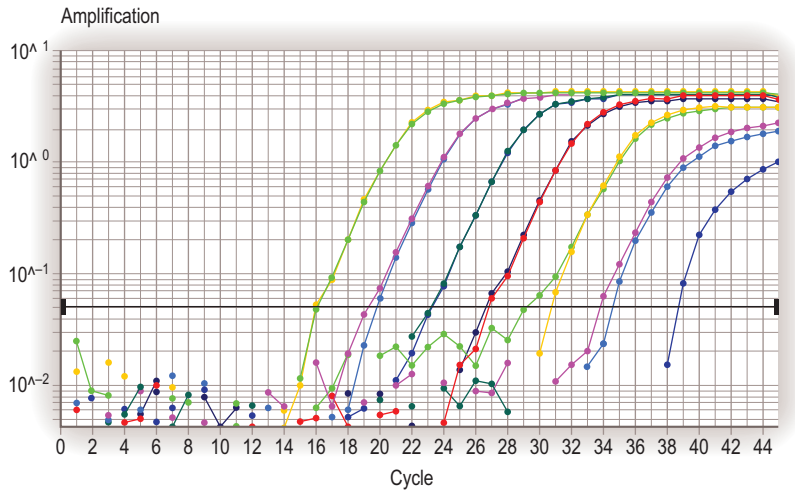


Fig. 3 **Amplification curves from a realtime polymerase chain reaction (PCR).**

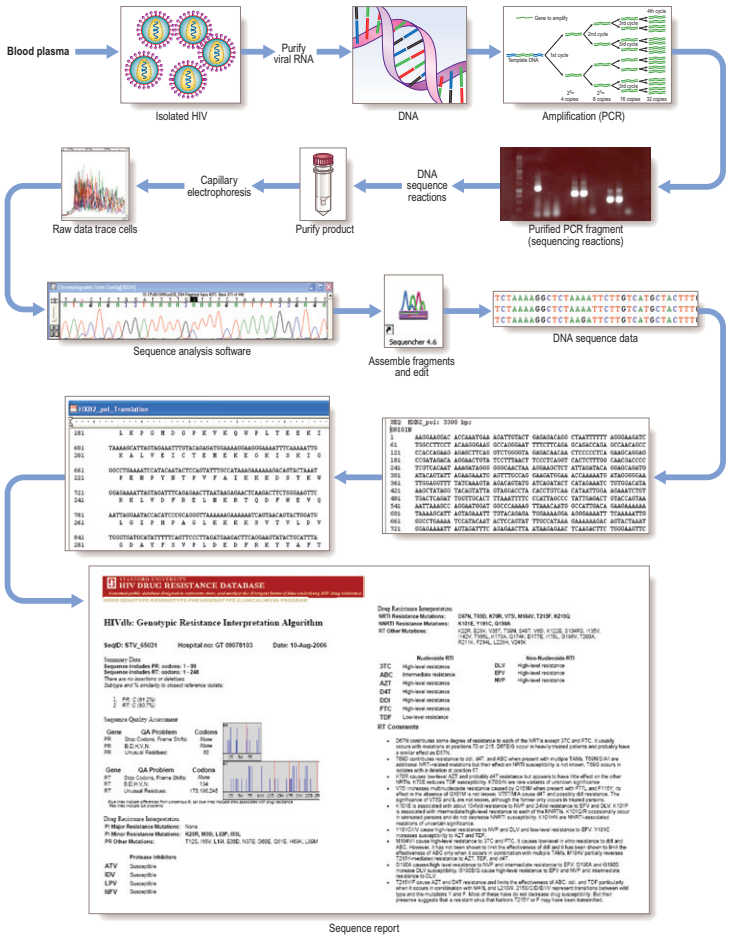


Fig. 4 **Genotypic antiretroviral drug resistance testing.** (Resistance report courtesy of Prof Shafer, Stanford University.)

- to estimate infectivity, e.g. the risk of mother-to-child transmission of HIV decreases with the maternal HIV viral load.

Finally, genotyping may be used as:

- prognostic marker, e.g. patients infected with hepatitis C virus genotype 1 are less likely to respond to antiviral treatment; infection with some types of HPV entails a high risk of developing cervical cancer
- therapeutic marker to monitor antiviral chemotherapy, e.g. HIV genotypic resistance testing to guide the choice of antiretroviral treatment regime (Fig. 4)
- to elucidate the source of infection, e.g. suspected transmission of HIV or HBV to sexual partners or patients.

Further reading

Berger A, Preiser W, Doerr HW: The role of viral load determination for the management of human immunodeficiency virus, hepatitis B virus and hepatitis C virus infection, *Journal of Clinical Virology* 20(1–2):23–30, 2001.

Disinfection and sterilisation

Introduction

Sterilisation and disinfection are important practices in the clinical and laboratory setting where viruses can be transmitted to humans. Surgical instruments, for example, should not be contaminated with pathogens when they are used in a procedure; similarly, the area to be cut open should be as clean as possible, and not contaminated with organisms.

Definitions

Sterilisation: the complete destruction of all viable microorganisms from a surface, including endospores.

Disinfection: the killing of microorganisms on a surface to the point where they no longer pose a threat of disease.

Antisepsis: the disinfection of human tissue.

Decontamination: removal of pathogenic organisms in order to allow safe handling.

Infection control

Infection control is an essential safety component of any setting where pathogens are present. In the clinical setting, it includes, for example, the proper use of antiseptics for wound care, clean practices when performing surgery, sterile equipment, safety when handling potentially infectious items such as used needles, and appropriate hand-washing before touching patients. In certain circumstances, protective clothing (Fig. 1) is required and gloves are essential when taking blood. In the laboratory setting, where specific organisms are deliberately cultured, it includes a wide range of methods to prevent transfer of those organisms to the individuals working with them.

Methods

Mechanical

The most basic mechanical method of disinfection is cleaning with soap and water to remove dirt, organic material such as vomitus or blood, and to start the disinfection process. Soap and detergents are able to damage certain cellular and viral membranes. Cleaning an object or surface (Fig. 2) is essential prior to more specific disinfection or sterilisation, which is not fully effective in the presence of dirt and dense organic matter. Ultrasound can be used to remove dirt where detergents and/or scrubbing are not suitable.

Filtration is a means by which large particles are removed from a solution. Depending on the size of the pores in the filter, different sized particles can be retained. Some filters are designed to filter out only gross debris, while some may filter out bacteria but allow viruses to pass through, and some may filter out even viruses when used in series, as in the case of the high-efficiency particulate air (HEPA) filter.

Fig. 1 **Healthcare worker preparing to enter an isolation facility for patients with highly infectious pathogens.** (Photo courtesy of Prof S Mehtar, University of Stellenbosch.)

Fig. 2 **Mechanical cleaning combined with disinfectants.** (Photo courtesy of CDC.)

Sterilisation of certain liquids by filtration can be achieved with filters in combination with agents that bind certain substances.

Heat

Heat is used in a variety of different ways:

- Autoclaves – these combine heat, steam and pressure to transmit heat energy to organisms in an optimal way in order to kill them. 121°C for at least 15 minutes is a commonly used protocol. This will kill all bacteria, protozoa, fungi and viruses, but will not completely inactivate prions (Fig. 3).

Fig. 3 **(A) Top-loading and (B) front-loading autoclaves. Different sizes are used, depending on the needs of the facility.**

Fig. 4 **SARS specimen kit – plastic items will not survive heat and require irradiation or ethylene oxide sterilisation.** (Photo courtesy of Prof S Mehtar, University of Stellenbosch.)

- Dry heat – items are heated to higher temperatures than autoclaves, as dry heat does not transfer energy to organisms as well as autoclaving.
- In laboratories, small items, such as metal loops used to inoculate agar plates with specimens from patients, can be heated until red hot.
- Boiling and cooking are effective for disinfection, but will not provide sterility.

Not everything can be heated – plastics, for instance, will melt (Fig. 4). Certain substances, such as powders, require dry heat, as do certain metals which could oxidise in an autoclave. Incineration is inappropriate for items meant for re-use, and is used to sterilise medical waste prior to discarding it.

Irradiation

X-rays and gamma rays can be used to sterilise objects, and this is often used for the sterilisation of disposable plastic items such as syringes or drip sets. The radiation damages DNA and protein structures in the organisms.

Ultraviolet light is used in TB clinics and biosafety cabinets to sterilise surfaces. In addition to DNA and protein damage, UV light can convert oxygen (O_2) to ozone (O_3), which is destructive to organisms.

Chemical

Some items are sensitive to chemicals and cannot safely be sterilised in this way (Table 1). It is important to note that most disinfectants are negatively affected by organic material, which should be washed away before disinfecting an item.

Table 1 **Chemicals used for sterilised and disinfection and their properties**

Chemical/chemical group	Description
Ethylene oxide	Toxic, flammable. Good for items that cannot be heated. Somewhat time consuming.
Ozone	Toxic, unstable, must be made on site. Used for sterilisation and surface disinfection.
Aldehydes (formaldehyde CH_2O, glutaraldehyde $C_5H_8O_2$)	Toxic, time consuming for sterilisation. Formaldehyde can be used as a gas or liquid.
Hydrogen peroxide (H_2O_2)	Used for disinfection and antisepsis; can also be used for sterilisation as a liquid or gas (known as gas plasma sterilisation.)
Sodium hypochlorite (NaClO)	Mainly used as a disinfectant, at a 1:10 dilution of commercial sodium hypochlorite solution, at which it is most active. Active component is hypochlorous acid. Hypobromites also used. Degrades in the presence of organic material.
Chlorine	Forms hypochlorous and hypochloric acids in water, which are active components.
Iodine	Used in many disinfectants and antiseptics. Lugol's iodine consists of iodine and potassium iodide.
Chlorine dioxide (ClO_2)	Explosive; commonly used as a water disinfectant.
Potassium permanganate ($KMnO_4$)	Used for disinfection and as an antiseptic. Has also been used as a topical antifungal/antibacterial agent.
Phenol	Also called carbolic acid. Phenols are commonly used in household disinfectants.
Quaternary ammonium compounds (QACs)	Benzalkonium chloride (BAC) is the most well-known. Relatively non-toxic and non-corrosive. Is used in hand towels, medical disinfectants.

Table 2 **Viruses and agents effective against them**

Virus	Env	Effective agents
Rabies	+	Heat, soap, non-ionic detergents, iodine, alcohol, hypochlorite, QACs, formaldehyde
Ebola/Marburg	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents, heat
Paramyxoviruses	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents, QACs, heat
Influenza	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents, phenols, QACs, heat
Lassa virus	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents
Bunyaviruses	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents
Enteroviruses	–	Hypochlorite, UV, formaldehyde, phenols
Rhinovirus	–	Hypochlorite, UV, formaldehyde, phenols
Hepatitis A	–	Hypochlorite, UV, formaldehyde, phenols
Hepatitis B	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents, QACs
Hepatitis C	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents
Rubella	+	Lipid solvents, non-ionic detergents, formalin, heat, pH
Herpes viruses	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents, QACs
HIV	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents
Coronaviruses	+	Lipid solvents, non-ionic detergents, formaldehyde, oxidising agents
Adenoviruses	–	Hypochlorite, chlorine
Rotavirus	–	Hypochlorite, chlorine
Pox viruses	+	Chlorine, hypochlorite, QACs, formaldehyde
Papillomaviruses	–	Hypochlorite, chlorine
Parvoviruses	–	Formaldehyde, oxidising agents, γ-radiation

Viruses and disinfection

The ability of an agent to inactivate a virus depends on the properties of the agent combined with the properties of the virus (Table 2). In general, viruses with lipid membranes are more labile, and are susceptible to lipid solvents (such as alcohol) and detergents which break up the membrane, whereas those viruses with no membrane are more hardy. The concentration of the agent used also determines its ability to render viruses harmless.

Transfusion and transplant safety

Various infectious agents can be transmitted through the inoculation of blood from viraemic (i.e. virus is present in the blood) donors. This mode of transmission is largely an iatrogenic one. Once blood transfusion and invasive procedures (that breach the patient's intact skin) had been introduced into clinical practice, this mode of transmission became relevant: either via transfusion of blood, or through needlestick injury, which may be regarded as 'unplanned micro-transfusion'.

Individuals suffering from an acute viral illness are infectious during the viraemic phase. This is notorious in patients with haemorrhagic fever who may be the origin of big health-care-associated outbreaks. A few cases have also been described of health-care staff becoming infected with dengue virus after exposure to the blood of a patient with dengue fever through needlestick injury. Likewise, blood donated during the acute phase of inapparent hepatitis A or West Nile virus infections has transmitted the infections to recipients. Whilst these are, overall, rare events (given the short-lived nature of viraemia in these acute viral infections), they have, nevertheless, in some cases necessitated the introduction of additional screening policies.

The main concern when it comes to transfusion safety is, however, posed by those viruses that cause persistent, chronic infection and are blood-borne. They are typically transmitted when asymptomatic, apparently healthy individuals donate blood for transfusion. Most notable in this context are hepatitis B (HBV) and hepatitis C (HCV) as well as human immunodeficiency viruses (HIV). In some parts of the world or in certain high-risk recipients, such as pregnant women or immunosuppressed transplant recipients, other viruses such as human T-cell lymphotropic viruses (HTLV-I and II), cytomegalovirus (CMV), parvovirus B19 and West Nile virus (WNV) may also be of relevance. Last but not least, the possibility of transmitting sub-viral agents causing prion diseases, most notably variant Creutzfeldt-Jakob disease (CJD), via blood has been recognised in recent years.

A number of steps are used to reduce the risk of transfusion-transmitted viral infections:

- Donor exclusion based on risk factors such as lifestyle, clinical history, etc. (see Box 1).
  When HIV first appeared in developed countries in the early 1980s and its transmissibility through blood had become known, the introduction of questionnaires to identify and exclude blood donors belonging to recognised risk groups was the most important step towards lowering the risk, even before screening tests became available.
- Screening for serological markers of infection.
  In developed countries, all donors are screened for antibodies to HIV and HCV as well as for HBV surface antigen. In certain parts of the world or for certain groups of recipients, such as pregnant women and immunocompromised transplant recipients, additional markers are added.
- Nucleic acid testing (NAT) for the direct, highly sensitive detection of infectious agents.
  Concerns about the possibility of missing recently acquired infections in patients still undergoing seroconversion (i.e. before specific antibodies had become detectable) led to the introduction of additional NAT-based screening in many developed countries. The enormous expense has, however, led to controversy regarding its cost effectiveness.

As a result, blood transfusions are now safer than ever before. However, the gap between the ideal standard as implemented in 'rich' countries and the reality in many developing countries, where even routine serological screening may not always be available, is widening further.

- Pathogen-reduction systems.
  Leukofiltration reduces the number of white blood cells, which carries certain advantages, including the elimination of cell-associated viruses such as CMV and HTLV.
- Pathogen inactivation technologies.
  Such methods promise to further reduce the residual risk of transfusion-acquired transmission of both known and, importantly, emerging viruses (Table 1). However, they must not interfere with the wanted properties of the blood which poses challenges.

Solid organ (such as kidney, liver, heart) and bone marrow transplant recipients are at increased risk of viral infections. The explanted organ as such is capable of harbouring certain viruses which can then infect the recipient. In addition, infections may be acquired from other sources (e.g. respiratory viruses).

This is aggravated by the fact that transplant recipients are iatrogenically immunocompromised, in order to reduce the risk of rejection. At the same time, this suppression of the immune system impairs the recipient's ability to effectively control infections. In addition to newly acquired viral infections, reactivation of latent viruses may occur and cause serious illness (e.g. herpesviruses).

Infection continues to be a major cause of mortality and morbidity in transplant recipients and, due to their increasing numbers, this problem is likely to grow further. Infections may

Table 1 **Contemporary risk of transmitting any of the blood-borne viruses for which screening is performed** (Adapted from Dodd 2009 and 2002.)

Virus	Risk per transfused unit	Transmission rate when an infected unit is transfused
HIV-1, HIV-2	1:2135000	90%
Hepatitis C	1:1935000	90%
Hepatitis B	1:205000	70%
HTLV	1:3000000	30%
West Nile virus*	1:10000 to 1:1000	Unknown
Parvovirus B19	1:40000 to 1:3000	Low
Hepatitis A and E	1:1000000	Low

*Prior to nucleic acid testing

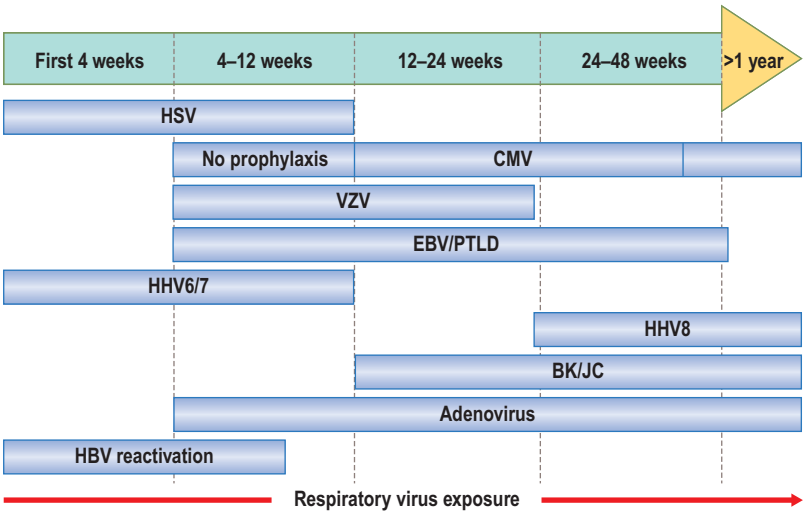


Fig. 1 **Timeline of post-transplant viral infections in solid organ transplant recipients.**

Box 1 **Screening of blood donors**

Risk assessment

Blood donation may transmit HIV, hepatitis B, hepatitis C, and other viruses from the donor to potential recipients. A detailed, relevant history is therefore taken from the donor prior to donating blood, in order to assess risk. Specific questions regarding sexual practices, medication, pregnancy and known illnesses are asked, with certain questions given a time frame, e.g. a new sexual partner within the last 6 months. The donor must often sign a declaration that he has answered the questions truthfully. A summary of the questions has been provided here to give an idea of the risk evaluation. Adapted from the donor questionnaire of the Western Province Blood Transfusion Service, South Africa:

Lifestyle-related questions

1. Athletic or gym programme
2. New sexual partners within the last 6 months
3. Receiving or giving money or drugs in exchange for sex
4. Recent sex between males
5. Recent sexually transmitted infections
6. Occupation (risk of dizziness after donation may be hazardous)
7. Needlestick injuries
8. Exposure to blood or body fluids.

Health-related questions

1. Current state of health and recent illnesses
2. HIV status of donor and sexual partners
3. Cardiovascular and pulmonary illness, diabetes, epilepsy, cancer and bleeding disorders
4. Pregnancy, miscarriage, abortion and breastfeeding
5. Chronic medication, certain specific medications, drug/vaccine trials, vaccinations
6. Malaria, TB, hepatitis, potential exposure to Creutzfeld-Jakob Disease
7. Tattoos, body piercing, drug use, acupuncture, ritual health practices
8. Recent blood transfusions, organ transplants, recent/scheduled surgery
9. Recently assaulted or stabbed.

have a direct effect on the graft or trigger rejection, may cause disseminated infection and a variety of often severe organ manifestations, may lead to post-transplant malignancies and may further impair host immunity.

Management strategies rely on screening of donor and recipient pre-donation as well as timely testing for infections in the post-transplant period (Fig. 1). As antiviral treatment, where available, is often unsuccessful once overt disease has become established, prophylactic administration of antiviral drugs may be preferable. Some drugs, however, have undesirable effects. In these cases, for example against CMV, so-called pre-emptive treatment strategies rely on regular monitoring of patients for evidence of active viral infection, with the aim of starting antiviral therapy before clinical disease develops.

Haematopoietic stem cell or bone marrow transplant recipients are a special case. They have received infusions of haematopoietic cells to re-establish marrow function that is damaged (often iatrogenically – to treat haematological malignancy) or defective. In allogeneic bone marrow transplantation, marrow is transferred from one person to another. Here the situation is somewhat opposite to a solid organ transplant: it is the recipient's body that may harbour various viruses and the donor's immune system that must attempt to control these. Thus, the transplantation of bone marrow from a CMV-negative donor into a CMV-positive, i.e. latently infected, recipient carries the highest risk of post-transplant CMV disease; with solid organs such as kidney it would be an organ from a positive donor being transplanted into a negative recipient.

Further reading

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Dodd RY, Notari EP 4th, Stramer SL: Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population, *Transfusion* 42(8):975–979, 2002.

Peggs KS, Preiser W, Kottaridis PD, et al: Extended routine polymerase chain reaction surveillance and pre-emptive antiviral therapy for cytomegalovirus after allogeneic transplantation, *British Journal of Haematology* 111(3):782–790, 2000.

Antiviral drugs – history and obstacles

History

More than 40 antiviral agents are currently available for the treatment of viral infections, including those caused by HIV, hepatitis B virus (HBV), hepatitius C virus (HCV), influenza and the herpesviruses. With increasing developments in molecular biology, a lot of new research are directed towards the design and development of new, specific drugs tailor-made to combat viral infections. Many of the older drugs were discovered incidentally. Dr Bill Prusoff (see Fig. 1) discovered the antiviral properties of idoxuridine, a drug active against herpes simplex (HSV), while trying to develop drugs to combat neoplastic disease. In 1977, one of the most useful antiviral drugs, acyclovir, was discovered during a drug development programme not primarily aimed at antiviral therapy. In the early 1980s HIV appeared as a major new viral force to be reckoned with, and in 1985 AZT (azidothymidine) proved to be the first specific inhibitor of the retroviral reverse transcriptase enzyme. Today, antiviral drug research primarily focuses on the design and development of new drugs and the investigation into new retroviral targets that will combat HIV/AIDS, the cause of a global pandemic, and to date still an incurable infection.

Fig. 1 **Prof. Bill Prusoff.** (Reproduced with permission from Prof. Prusoff, Yale University.)

Antivirals – not all that they seem to be?

Although the treatment of viral diseases has come a long way, **obstacles** with regard to the use of antiviral drugs are still encountered. Here are a few:

- Many antiviral agents do not show sufficient selectivity to the viruses that they target. Host cells are also affected by the toxic effects of these drugs (e.g. many antiretrovirals).
- Some viruses have multiple serotypes (e.g. rhinoviruses) or constantly change their antigenic determinants (e.g. influenza virus and HIV). These changes may affect the target molecules of antiviral drugs.
- Many viruses cause latent infections that result in a general decrease in gene expression and expression of drug targets.
- Antiviral drugs are mostly virostatic. Complete inhibition of viral replication seldom occurs. This results in viral persistence and may even give rise to drug resistant mutants!
- Many viruses are impossible or very difficult and impractical to culture (e.g. human papilloma virus, and hepatitis B and C viruses). This complicates experimental studies aimed at the development of new drugs against these viruses.
- Most viral infections are acute and short-lived, with cessation of viral replication and the possibility of drug intervention at the time of diagnosis. (e.g. influenza, hepatitis A).
- Some infections are mild and self-limiting and do not warrant extensive research to develop expensive drugs to treat them with. Good examples include the common cold (caused by rhinoviruses or coronaviruses) and childhood diseases like roseola, parvovirus B19 infection and mumps.

Table 1 **Agents active against the herpesviruses**

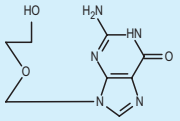
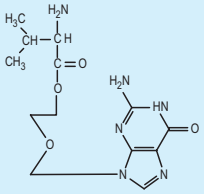
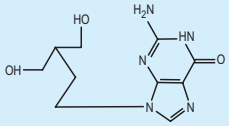
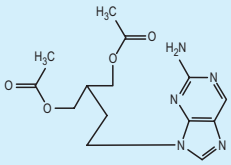
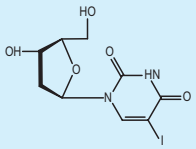
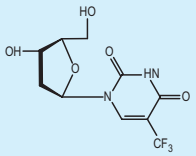
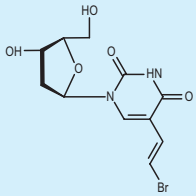
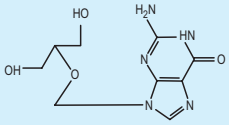
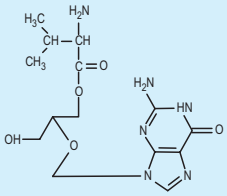
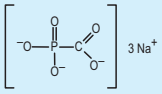
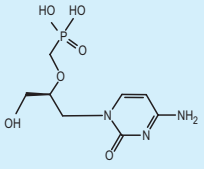
Drug	Structure	Trade name	Spectrum of activity	Principal indication(s)	Route(s) of administration
■ Acyclovir		Zovirax®	HSV-1, HSV-2, VZV	Mucosal, cutaneous and systemic herpes simplex virus (HSV)-1 and HSV-2 infections (e.g. HSV keratitis, HSV encephalitis, herpes labiales, genital herpes, neonatal herpes) Vericellar zoster virus (VZV) infections (incl. herpes zoster)	Oral, topical, intravenous
■ Valaciclovir		Zelitrex® Valtrex®	As for acyclovir	As for acyclovir Prophylaxes for cytomegalovirus (CMV) infections in transplant recipients	Oral
■ Penciclovir		Denavir® Vectavir®	As for acyclovir	Mucocutaneous HSV infections, especially herpes labialis	Topical
■ Famciclovir		Famvir®	As for acyclovir	As for acyclovir	Oral
■ Idoxuridine		Herpid® Idoxene®	HSV-1, HSV-2, VZV	HSV keratitis	Topical
■ Trifluridine		Viroptic®	HSV-1, HSV-2, VZV	HSV keratitis	Topical
■ Brivudin		Zostex® Zonavir®	HSV-1 and VZV	HSV-1 keratitis and herpes labialis VZV infections, especially herpes zoster	Oral, topical
■ Ganciclovir		Cymevene® Cytovene®	HSV-1, HSV-2, CMV	Treatment and prophylaxis of CMV infections (e.g. CMV retinitis) in immunocompromised/AIDS patients).	Oral, intravenous, intraocular/ intravitreal implant
■ Valganciclovir		Valcyte®	As for ganciclovir	As for ganciclovir	Oral
■ Foscarnet		Foscavir®	HSV-1, HSV-2, VZV, CMV, HIV	CMV retinitis in AIDS patients, acyclovir-resistant mucocutaneous HSV and VZV infections in immunocompromised patients	Intravenous infusion
■ Cidofovir		Vistide® Forvade®	HSV-1, HSV-2, VZV, CMV, HPV, polyomaviruses, adenoviruses, poxviruses	CMV retinitis in AIDS patients acyclovir-resistant mucocutaneous HSV infections in immunocompromised patients Recurrent genital herpes, genital warts, laryngeal, cutaneous and cervical HPV lesions Molluscum contagiosum and orf lesions Adenovirus infections PML (progressive multifocal leukoencephalopathy)	Intravenous infusion, topical
■ Fomivirsen	5′-GCG TTT GCT CTT CTT CTT GCG-3′	Vitravene®	CMV	CMV retinitis in AIDS patients	Intraocular (intravitreal)

Table 2 **Agents active against influenza viruses**

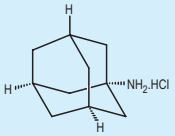
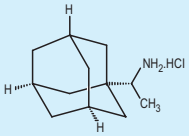
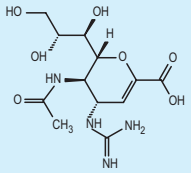
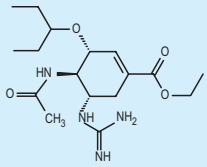
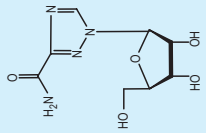
Drug	Structure	Trade name	Spectrum of activity	Principal indication(s)	Route(s) of administration
■ Amantadine		Symmetral® Amantan®	Influenza A	Early treatment (within 48 hours) and prophylaxis of influenza A Treatment of Parkinson's disease	Oral
■ Rimantadine		Flumadine®	Influenza A	Early treatment (within 48 hours) and prophylaxis of influenza A	Oral
■ Zanamivir		Relenza®	Influenza A and B	Early treatment (within 48 hours) of influenza A and B	Inhalation
■ Oseltamivir		Tamiflu®	Influenza A and B	Early treatment (within 48 hours) and prophylaxis for influenza A and B	Oral
■ Ribavirin		Virazole® Virazid®	Broad range of DNA and RNA viruses including influenza A and B, respiratory syncytial virus (RSV), hepatitis C virus (HCV), measles, Lassa, Junin, etc.	Treatment of RSV in high-risk infants (by aerosol) Treatment of HCV in combination with IFN alpha (Rebetron®) or pegylated IFN alpha	Oral (for treatment of HCV) Aerosol (for treatment of RSV) Intravenous for treatment of Lassa fever

Table 3 **Agents active against hepatitis B**

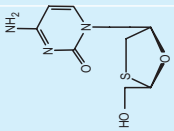
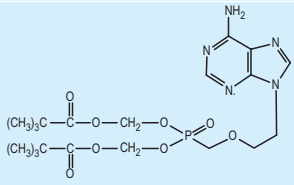
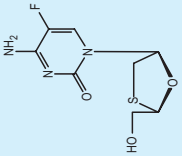
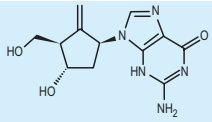
Drug	Structure	Trade name	Spectrum of activity	Principal indication(s)	Route(s) of administration
■ Human recombinant interferon alpha		Intron®	Hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), human herpesvirus (HHV) 8	Treatment of chronic hepatitis B Prevention of HBV reactivation in stages of immunosuppression Treatment of HCV in combination with ribavirin	Subcutaneous Intramuscularly Intravenous
■ Pegylated interferon alpha		Peg-Intron® Pegasys®	HBV, HCV, HPV, HHV 8	As for standard interferon Pegylation allows for slower renal clearance, better tissue distribution and a longer half-life of the drug	As for standard interferon
■ Lamivudine /3TC		Epivir® Zeffix®	HBV, HIV-1 and HIV-2	Treatment of chronic hepatitis B Treatment of HIV infection. Part of combination therapy, e.g. with AZT (Combivir®), with AZT and ABC (Trizivir®)	Oral
■ Adefovir dipivoxil		Hepsera®	HBV, HIV, herpes simplex virus (HSV), cytomegalovirus (CMV)	Treatment of drug (lamivudine) resistant chronic HBV	Oral
■ Emtricitabine / FTC		Emtriva®	HIV, possibly also HBV	Treatment of HIV infections Pursued for treatment of HBV infection.	Oral
■ Entecavir		Baraclude®	HBV	Treatment of drug (lamivudine) resistant chronic HBV	Oral

Table 4 **Agents active against picornaviruses**

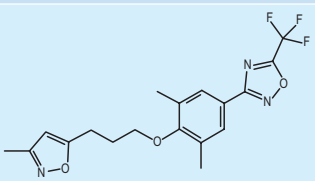
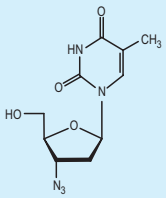
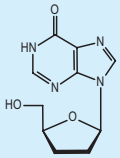
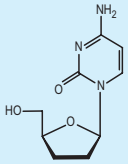
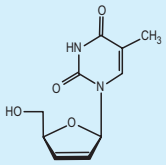
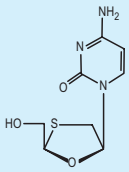
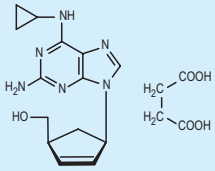
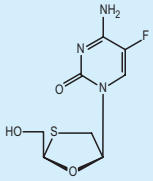
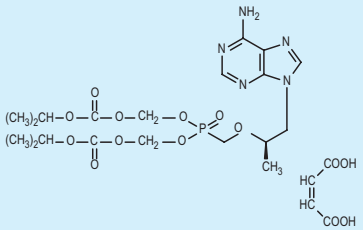
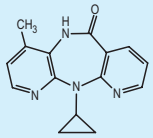
Drug	Structure	Trade name	Spectrum of activity	Route(s) of administration
■ Pleconaril		Picovir®	Picornaviruses (enteroviruses and rhinoviruses)	Oral Intranasal

Table 5 **Agents active against HIV**

Drug	Structure	Trade name	Spectrum of activity	Principal indication(s)	Route(s) of administration
Nucleoside reverse transcriptase inhibitors					
■ Zidovudine /AZT		Retrovir®	HIV (types 1 and 2)	HIV-1 infection, in combination with other drugs.	Oral
■ Didanosine /ddI		Videx®	As for zidovudine/ AZT	As for zidovudine/ AZT	Oral
■ Zalcitabine /ddC		Hivid®	As for zidovudine/ AZT	As for zidovudine/ AZT	Oral
■ Stavudine/ d4T		Zerit®	As for zidovudine/ AZT	As for zidovudine/ AZT	Oral
■ Lamivudine /3TC		Epivir® Zeffix®	HBV, HIV 1 and 2	Treatment of chronic hepatitis B. Treatment of HIV infection. Part of combination therapy, e.g. with AZT (Combivir®), with AZT and ABC (Trizivir®)	Oral
■ Abacavir /ABC		Ziagen®	As for zidovudine/ AZT	As for zidovudine/ AZT	Oral
■ Emtricitabine /FTC		Emtriva®	HIV, possibly also hepatitis B virus (HBV)	Treatment of HIV infections Pursued for treatment of HBV infection	Oral
Nucleotide reverse transcriptase inhibitors					
■ Tenofovir disoproxil		Viread®	HIV 1 and 2, HBV	HIV-1 infection, in combination with other drugs	Oral
Non-nucleoside reverse transcriptase inhibitors					
■ Nevirapine		Viramune®	HIV-1	HIV-1 infection, in combination with other drugs.	Oral

Continued

Table 5 **Agents active against HIV—cont'd**

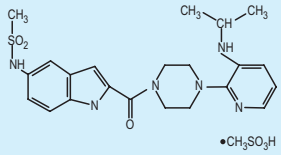
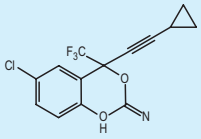
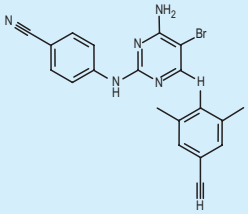
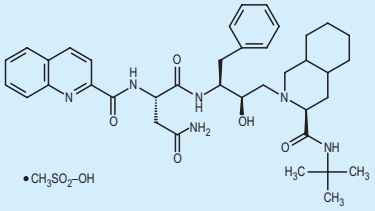
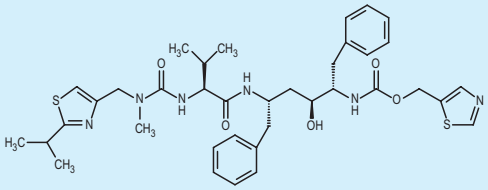
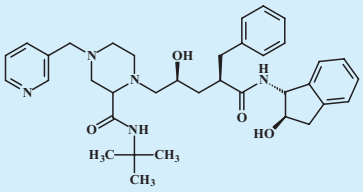
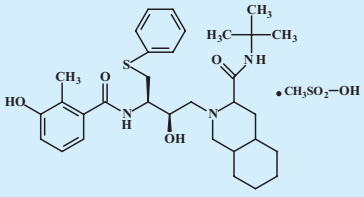
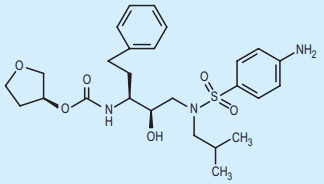
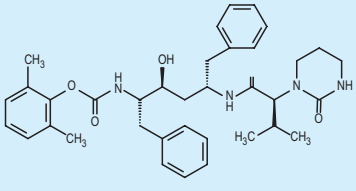
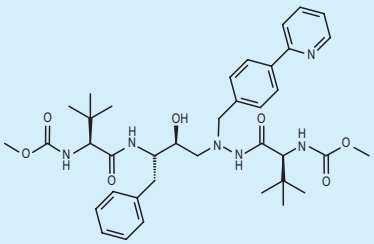
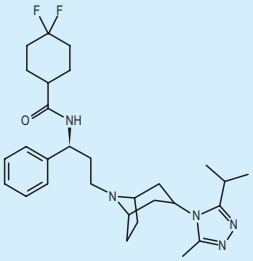
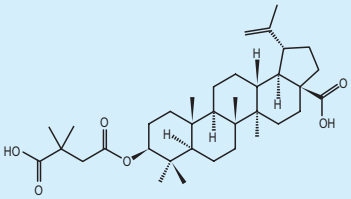
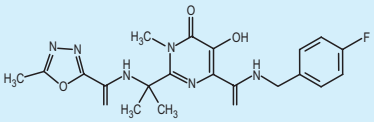
Drug	Structure	Trade name	Spectrum of activity	Principal indication(s)	Route(s) of administration
■ Delavirdine		Rescriptor®	HIV-1	HIV-1 infection, in combination with other drugs.	Oral
■ Efavirenz		Stocrin® Sustiva®	HIV-1	HIV-1 infection, in combination with other drugs.	Oral
■ Etravirine		Intelence®	HIV-1	HIV-1 infection, in combination with other drugs. Usually salvage therapy.	Oral
Protease inhibitors					
■ Saquinavir		Invirase®	HIV-1 and HIV-2	HIV-1 infection, in combination with other drugs	Oral
■ Ritonavir		Norvir®	HIV 1 and 2	As for saquinavir	Oral
■ Indinavir		Crixivan®	HIV 1 and 2	As for saquinavir	Oral
■ Nelfinavir		Viracept®	HIV 1 and 2	As for saquinavir	Oral
■ Amprenavir		Agenerase®	HIV 1 and 2	As for saquinavir	Oral
■ Lopinavir		Kaletra® (combined with ritonavir)	HIV 1 and 2	As for saquinavir	Oral

Table 5 **Agents active against HIV—cont'd**

Drug	Structure	Trade name	Spectrum of activity	Principal indication(s)	Route(s) of administration
■ Atazanavir		Reyataz®	HIV 1 and 2	As for saquinavir	Oral
CCR5 entry inhibitors					
■ Maraviroc		Selzentry®/ Celsentri®	HIV-1	HIV-1 infection, in combination with other drugs, where the virus uses the CCR5 co-receptor	Oral
Maturation inhibitors					
■ Bevirimat		–	HIV-1	In clinical trials	Oral
Integrase inhibitors					
■ Raltegravir		Isentress®	HIV-1	HIV-1 infection, in combination with other drugs	Oral
Viral entry inhibitors					
■ Enfuvirtide /T-20	YTSLIHSLIEE-SQNQQEKNE-QELLELDKW-ASLWNWF	Fuzeon®	HIV-1	HIV-1 infection, in combination with other drugs Usually salvage therapy	Subcutaneous injection

Antiviral drugs – resistance development

Table 6 **Antiviral drugs – resistance development**

Virus	Resistant drug(s)	Mechanism(s) for acquiring resistance	Alternative therapies
Herpes simplex virus (HSV) and varicella zoster virus (VZV) (especially in prolonged treatment of immunocompromised patients)	Acyclovir, valacyclovir, pencyclovir, famcyclovir	TK (thymidine kinase) mutation(s)	Foscarnet Cidofovir
HSV and VZV	Foscarnet, cidofovir	DNA polymerase mutation(s)	Possibly acyclovir, if not multidrug resistant
Cytomegalovirus (CMV)	Gancyclovir	UL 97 (phosphotransferase) mutation(s)	Foscarnet Cidofovir
CMV	Foscarnet, cidofovir	UL 54/DNA polymerase mutation(s)	Possibly gancyclovir, if not multidrug resistant
Influenza A	Amantadine, rimantadine	Mutation(s) in M2 protein channel	Neuraminidase inhibitors (zanamivir, oseltamivir), ribavirin
Hepatitis B virus (HBV)	Lamivudine/3TC	Mutation(s) in the YMDD motif of HBV DNA polymerase	Adefovir dipivoxil, entecavir
HIV	Nucleotide reverse transcriptase inhibitors (NRTIs)	↓ Incorporation of NRTI in DNA chain (e.g. M184V mutation that causes high level lamivudine resistance) Removal of NRTI from DNA chain (e.g. thymidine analogue mutations that confer resistance against AZT, D4T or tenofovir)	Other drug classes, e.g. protease inhibitors (PIs) and/or NNRTIs. In some scenarios, certain NRTIs may still be useful/active
HIV	Non-nucleotide reverse transcriptase inhibitors (NNRTIs)	Mutations that cause a spacial distortion of the reverse transcriptase enzyme and change the flexibility of the enzyme. This results in ↓ affinity of the hydrophobic pocket for NNRTIs (e.g. K103N and Y181C)	Cross-resistance is common amongst the NNRTIs. Other drug classes, e.g. NRTIs and/or PIs may be useful
HIV	PI	Mutation(s) in the catalytic site of the viral protease (e.g. V82A)	Cross-resistance is common amongst the PIs. Other drug classes, e.g. NRTIs and/or NNRTIs may be useful
HIV	Fusion inhibitor (enfuvirtide)	gp 41 or gp 120 mutation(s)	Other drug classes, e.g. NRTIs and/or NNRTIs and/or PIs may be useful

Antiviral drugs – modes of action

1. Neutralising antibodies against free virus (pre-replication)

Examples are: IVIg (intravenous immunoglobulin), HNIG (normal human immunoglobulin) and IMIg (intramuscular immunoglobulin).

Available for:
IVIg – varicella zoster virus (VZV), hepatitis B (HBV), rabies, cytomegalovirus (CMV), respiratory syncytial virus (RSV), and hepatitis A (HAV)

HNIG – measles, rubella, HAV

IMIg – RSV (palivizumab)

2. Decoy receptors

These are soluble receptors or receptor analogues that bind to the virus and prevents entry into the cell (e.g. soluble CD4 that binds to gp120 and prevents binding of gp120 to cellular CD4). Future developments may result in clinically useful drugs.

3. Entry inhibitors: blocking/inhibition of attachment or adsorption

An example is the CCR5 blocker, maraviroc. (CCR5 is a major co-receptor for binding to gp120 of HIV M-tropic strains). Another example is ibalizumab (a monoclonal antibody that binds to CD4 and prevents HIV from binding).

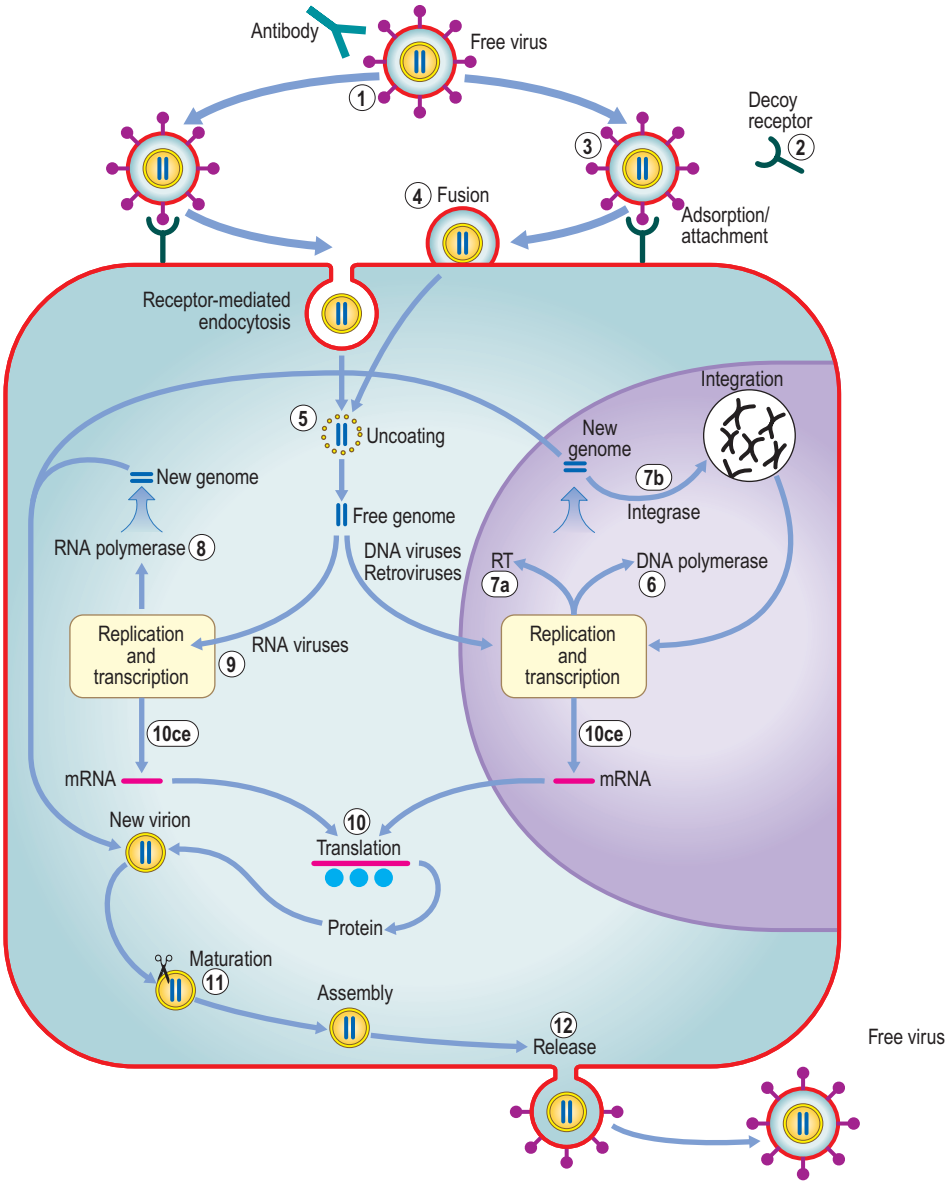


Fig. 1 **Antiviral targets.**

4. Entry inhibitors: inhibition of fusion

This process involves the inhibition of virus-cell fusion through coil–coil interaction with a region in gp41 of HIV-1, e.g. enfuvirtide.

5. Blocking/inhibition of uncoating

Examples include amantadine and rimantadine for influenza A. They block M2 ion channels, which are necessary for the passage of H^+ ions (acidity) needed for the decapsidation (uncoating) of the virus. Another blocking drug is pleconaril, which blocks the uncoating process of picornaviruses by interfering with the hydrophobic pocket structure on the viral capsid.

6. Inhibition of replication: DNA viruses

This involves the inhibition of DNA polymerase.

Nucleoside (guanosine) analogues – (Fig. 2)

Acyclovir – HSV-1 and 2 (mucosal, cutaneous, systemic) and VZV

Valacyclovir – HSV-1 and 2, VZV, CMV prophylaxis

Penciclovir – HSV (mucosal)

Famciclovir – HSV-1 and 2, VZV

Ganciclovir – CMV

Valganciclovir – CMV, adenovirus, vaccinia.

Other nucleoside analogues include:

Idoxuridine (mode of action) – HSV keratitis

Trifluridine (mode of action) – HSV-1 and 2, VZV

Brivudin (mode of action) – HSV, VZV.

Nucleotide analogues

Cidofovir (cytosine analogue) – HSV-1 and 2, VZV, CMV, papilloma-, polyoma-, adeno- and poxviruses (Fig. 3).

Pyrophosphate analogues

These interfere with pyrophosphate (diphosphate) binding to the viral DNA polymerase. They inhibit the formation of phosphodiester bonds between nucleotides (e.g. foscarnet – HSV 1, 2, VZV, HIV).

7. Inhibition of replication: retroviruses

a. Inhibition of the reverse transcriptase (RT) enzyme

Nucleoside and nucleotide analogues

Nucleoside analogues (NRTIs) (Fig. 4):

Zidovudine (AZT) –HIV

Didanosine (ddI) – HIV

Zalcitabine (ddC) – HIV

Stavudine (d4T) – HIV

Abacavir (ABC) – HIV

Lamivudine (3TC) – HIV, hepatitis B

Emtricitabine (FTC) – HIV, hepatitis B

Entecavir – hepatitis B

Nucleotide analogues (NtRTIs) (Fig. 5):

Tenofovir disoproxil – HIV, hepatitis B

Adefovir dipivoxil – HIV, hepatitis B

Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) bind to the allosteric 'pocket' (non-substrate binding site) of HIV RT, causing a conformational change in the active substrate-binding site of the enzyme, resulting in inhibition of the RT. Examples include:

Nevirapine

Efavirenz

Etravirine

b. Inhibition of the integrase enzyme

The drug binds to the integrase enzyme, preventing it from integrating proviral DNA into the host genome, e.g. raltegravir.

8. Inhibition of replication: RNA viruses

Inhibition of RNA polymerase

Drugs in this class (e.g. Ribavirin) have a broad spectrum of activity – influenza, parainfluenza, RSV, rhinovirus, mumps, measles, vaccinia, hepatitis C, CCHF, Lassa virus, hantavirus (Fig. 6).

9. Inhibition of transcription

For example, Ribavirin (see Fig. 6).

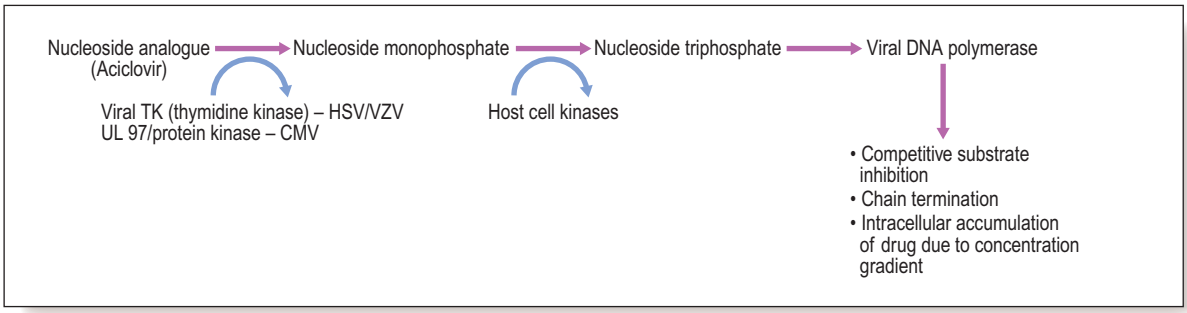


Fig. 2 **Nucleoside (guanosine) analogues – mode of action.**

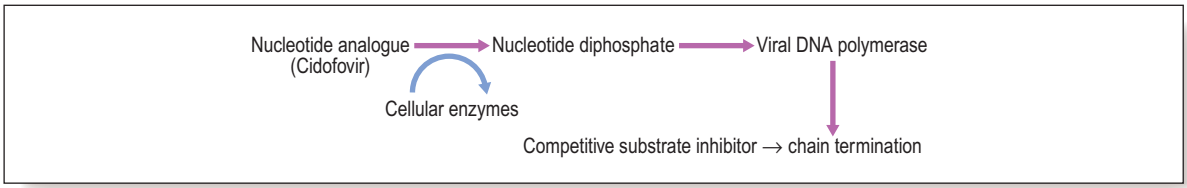


Fig. 3 **Cidofovir (cytosine analogue) – HSV-1 and 2, VZV, CMV, papilloma-, polyoma-, adeno- and poxviruses.**

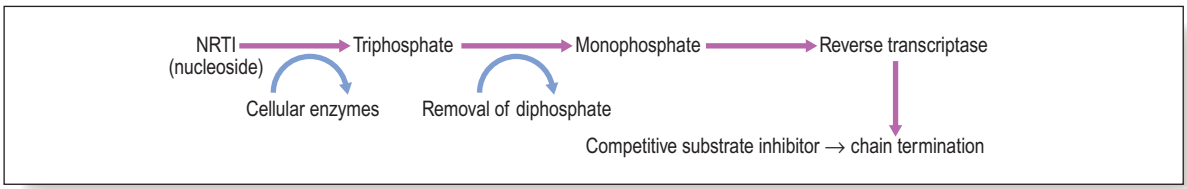


Fig. 4 **Nucleoside analogues (NRTI).**

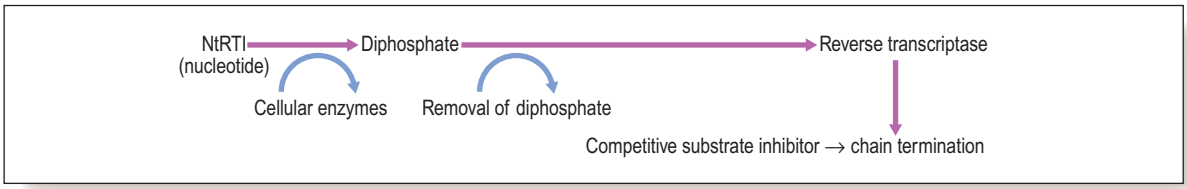


Fig. 5 **Nucleotide analogues (NtRTI).**

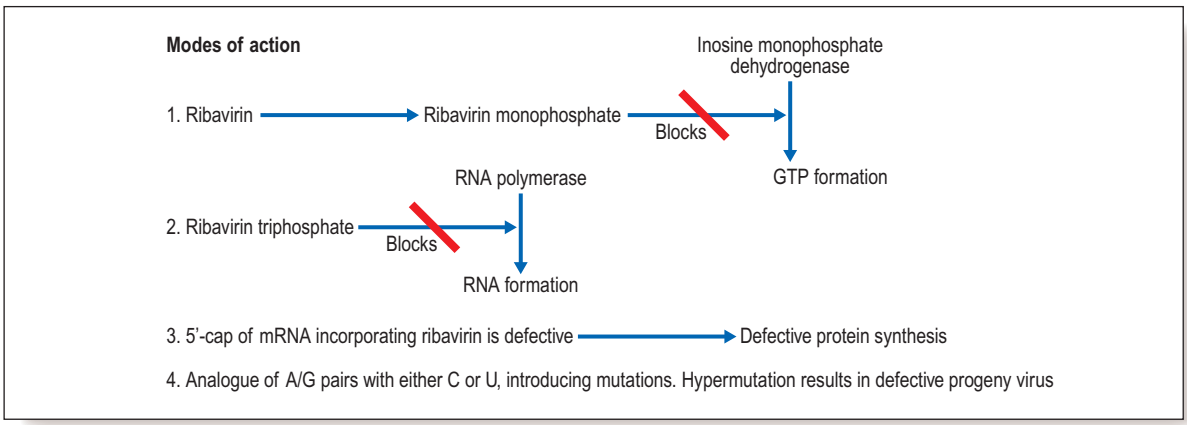


Fig. 6 **Spectrum of activity – ribavirin.**

10. Blocking/inhibition of translation/protein synthesis

a. Antisense DNA molecules

These hybridise to RNA, and prevent translation into proteins, e.g. fomivirsen (which has been used to treat CMV retinitis in the past). It is complementary to CMV immediate early 2 (IE2) mRNA. It hybridises to the transcript and inhibits translation of mRNA.

b. Antisense RNA molecules: experimental

These hybridise to RNA and induce breakdown of the RNA by cellular enzymes.

c. Ribozymes: experimental

These are strands of RNA that have a sequence with catalytic activity. The ribozyme RNA cleaves target RNA, leaving it non-functional.

d. Interferon alpha (Fig. 7)

This is a recombinant glycoprotein that induces an 'antiviral state' in uninfected neighbouring cells in response to various viruses. It is indicated for the treatment of chronic hepatitis B or hepatitis C infection, HPV, HHV8.

e. Ribavirin

This results in a damaged mRNA 5'-cap, thus preventing translation (Fig. 3).

11. Blocking/inhibition of maturation

a. Protease inhibitors (PIs)

They inhibit viral maturation by binding to protease. HIV protease cleaves the HIV GAG-POL precursor polyprotein and ensures that the new virions are mature and infective. HIV protease inhibitors include: saquinavir, ritonavir, amprenavir, indinavir, lopinavir, nelfinavir and atazanavir.

b. Maturation inhibitors

They bind to the GAG-POL precursor protein and thereby prevent HIV protease activity, e.g. bevirimat, vivecon – both experimental at present.

12. Inhibitors of viral release

Neuraminidase inhibitors for Influenza A and B

N-acetylneuraminic acid analogues. Inhibit viral neuraminidase (important for release) and trap the newly formed virions in the infected host cell e.g.

Zanamivir

Oseltamivir

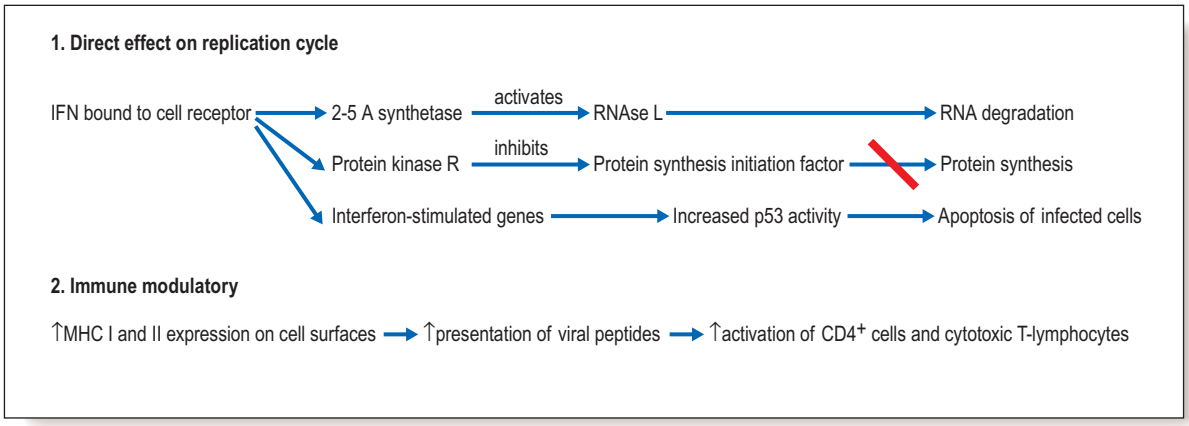


Fig. 7 **Interferon alpha – method of action.**

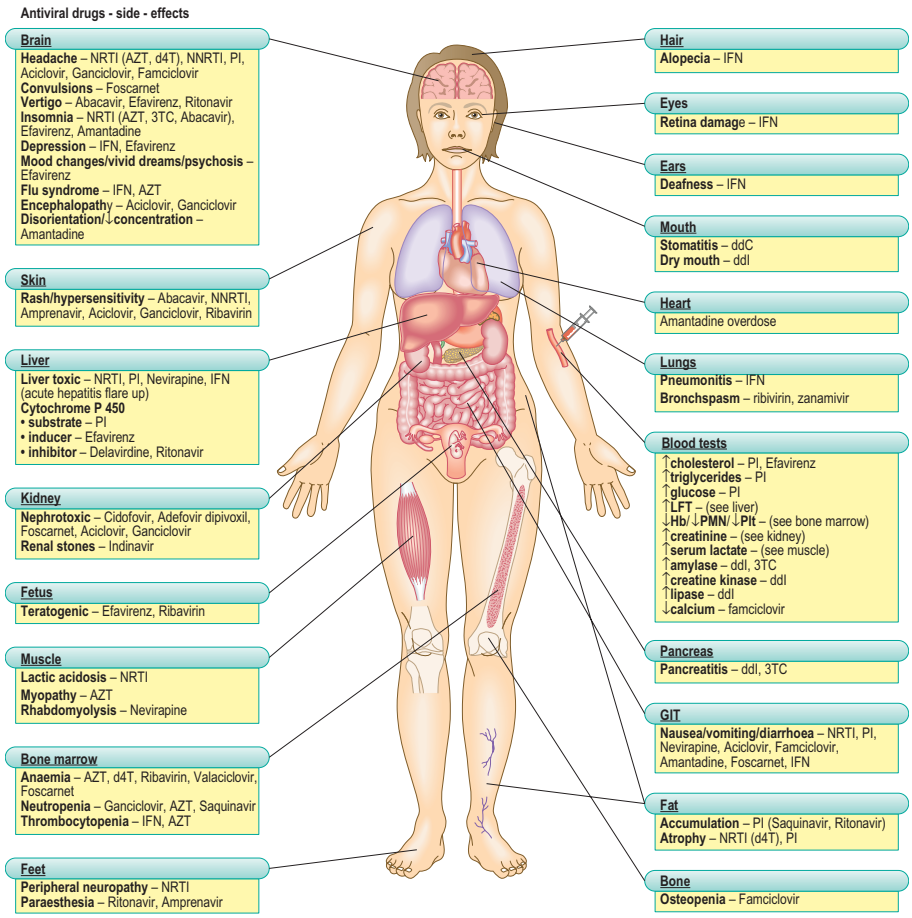


Fig. 8 **Antiviral drugs – side effects.**

Immunotherapy and immunoprophylaxis – passive and active immunity

Introduction

The human immune system provides protection against foreign antigens, many of which take the form of infectious agents such as bacteria, viruses, parasites and fungi. However, until the body has been exposed to an organism, it has to rely on innate immune responses for protection. Once exposed to an organism, the adaptive immune system is able to form a specific immune response and form memory cells able to respond more rapidly to future exposure to the organism.

The timing however is not always convenient – for protection, the individual needs to have an immune response ready before exposure, as exposure to the organism may result in disease. By exposing an individual to a harmless form of the organism's antigens, a similar immune response can be elicited without risk of disease resulting from a true infection. This is what occurs during active immunisation or vaccination – vaccines are non-pathogenic forms of the organism or its antigens. The result is ideally a combination of cellular and humoral immune responses that mimic those that occur with a natural infection. The aim is to produce either sterilising immunity (protection against infection by neutralising the organism before it can spread in the body) or protective immunity (protection against disease by halting the infection before pathology or symptoms develop).

Specific immunity can also be transferred to an individual without that individual developing an adaptive memory response. This occurs naturally when antibodies are transferred across the placenta from a mother to her child, and in clinical practice it can occur artificially by injecting antibodies into an individual. Passive transfer of cellular immunity is not routinely practised; however, HLA-compatible T-cells, including T-cells from the individual that have been stimulated in vitro, have been transfused into patients to provide an immune response against organisms or tumours.

Clinical uses for vaccines

Pre-exposure prophylaxis – vaccines taken before exposure may prevent infection or disease when the person is exposed to the wildtype virus.

Post-exposure prophylaxis – after exposure, vaccination may stimulate a more rapid immune response, providing protection. For detailed examples, see the sections on hepatitis B and rabies, as well as the section on post-exposure prophylaxis.

Therapy – therapeutic viral vaccines are still experimental, but scientists are interested in the potential effects on HIV progression.

Passive immunisation

Passive immunity is transferred from mother to child across the placenta, providing immunity for the first months of life. For the same reason, testing for IgG in infants may merely indicate maternal IgG, and not an acquired immunity in the infant. Also, response to certain vaccines may be sub-optimal as a result of these antibodies.

Historically, the first antibodies to be used clinically were against tetanus and diphtheria in 1890. During World War II, plasma protein fractionation was used to separate out antibodies, and eventually organism-specific antibodies could be separated from the rest. Viral infections were initially often treated with antibodies. Today antibodies – either normal human immunoglobulin (total immunoglobulin) or hyperimmune globulin (organism-specific) – are still an important part of prophylaxis against certain viral infections such as hepatitis A, hepatitis B, rabies, measles, varicella and respiratory syncytial virus (RSV). In the modern era of laboratory-synthesised monoclonal antibodies, RSV can be prevented with palivizumab, a recombinant human-mouse monoclonal, and monoclonal antibodies for other viruses, such as foravirumab and rafivirumab for rabies, are in development. Passive immunity has also shown some effect in preventing mortality in cases of Ebola.

Active immunisation

Vaccines are biological substances that are used to induce or improve immunity to a specific disease. The word vaccine comes from the Latin word for cow, i.e. *vacca*, via the Latin for *cowpox*, which Edward Jenner used to make his smallpox vaccine in 1796. Vaccines can be prophylactic (preventing disease) or therapeutic (ameliorating disease.) Therapeutic and preventative HIV vaccines are currently in clinical trials.

History of vaccines

Variolation, the practice of infecting people with low doses of smallpox, dates back to 1000 BC in India. It would generally induce a mild form of the disease, which would prevent the person from being re-infected. Edward Jenner realised that a milkmaid infected with cowpox would not subsequently get smallpox. Cowpox caused a mild infection, and so the first live vaccine was found. Later it was discovered that the virus being used for smallpox vaccination (Fig. 1) was in fact vaccinia, not cowpox, albeit related. Louis Pasteur (Fig. 2) went further with this process and developed the first rabies vaccine.

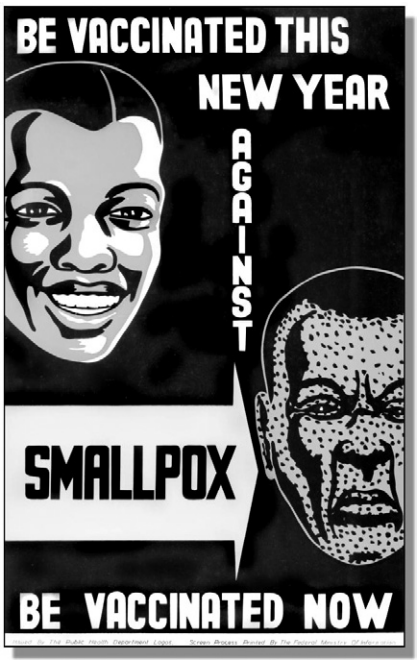


Fig. 1 **Advertisement for the smallpox vaccine.** (Photo courtesy of CDC/Stafford Smith.)

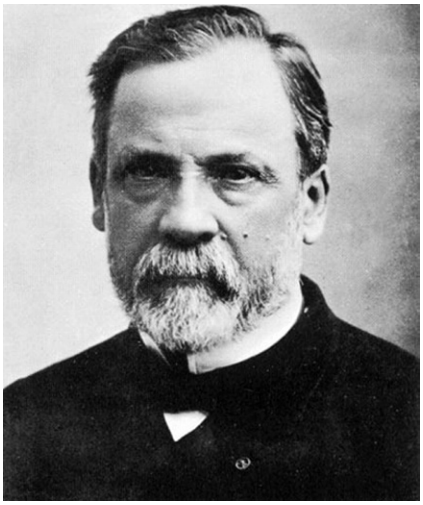


Fig. 2 **Louis Pasteur (1822–1895)** taken in 1878 by Gaspard-Félix Tournachon.

Types of vaccines

Several types of vaccines, and potential vaccines, exist, ranging from live organisms to DNA that results in the production of certain proteins made by the organism. See Table 1 for examples:

Live attenuated vaccines – a virus is passaged many times in a cell line, and this results in attenuation of the virus. Most live attenuated viruses carry little risk of serious infection, with the smallpox vaccine being the notable exception. Measles, varicella, oral polio and yellow fever vaccines may rarely induce complications similar to that of the wildtype virus.

Inactivated vaccines – wildtype viruses can be inactivated, usually with formaldehyde or a similar chemical, and thereafter no longer cause disease, although a protective immune response can still be formed against the virus.

Split vaccines – after inactivation of the viruses, the killed viruses are disrupted by detergents and used for vaccination.

Subunit vaccines – after inactivation of the viruses, killed viruses are then split into their many components and certain components are separated from the rest for use in the vaccine.

Recombinant vaccines – if a specific viral antigen or set of viral antigens can be identified, the genes for those antigens can be inserted into other organisms, e.g. brewer's yeast (*Saccharomyces cerevisiae*) in the case of hepatitis B surface antigen. In the case of human papillomavirus vaccines, virus-like particles (VLPs) are produced without any genome present, which means that they cannot replicate.

Table 1 **Types of vaccines and examples of each**

Viral vaccines			
LAV	***Whole killed***	***Subunit***	***Recombinant***
Varicella	Hepatitis A	Influenza	Hepatitis B
Measles		Hepatitis B†	HPV: VLPs
Mumps	Polio (IPV)		
Rubella	Rabies		
Polio (OPV)	Japanese encephalitis B		
Yellow fever	Tick-borne encephalitis		
Influenza (nasal)	Influenza		
Rotavirus	RVF*		
Smallpox*			

*Not publicly available; †limited availability. HPV, human papilloma virus; RVF, Rift Valley fever; VLP, virus-like particles; IPV, inactivated polio vaccine; OPV, oral polio vaccine.

Fig. 3 **Children lined up for their vaccines.** (Photo courtesy of CDC.)

Toxoid vaccines – if an organism produces a toxin, this toxin can be inactivated (thereby becoming a toxoid), and may still induce immunity that is protective against the toxin itself. No viral toxoid vaccines are available.

DNA vaccines – naked DNA may be injected, or delivered in other ways, into the skin or muscle. This DNA is taken up by the cells, and the proteins it codes for may be produced. Immunity against viral proteins can be induced in this way. No DNA vaccines are currently available commercially.

Vector vaccines – these are live but harmless viruses (or bacteria) that carry genes for other viruses, and are able to produce some of the proteins made by the harmful virus without risk of infection from those viruses. The vector vaccines may or may not be able to replicate completely in the host. Research into HIV vaccines has used vectors such as MVA (modified vaccinia Ankara) and canarypox.

Adjuvants – adjuvants are substances that enhance an immune response to a foreign protein. Without an adjuvant, the immune response may be weak or temporary, but the addition of an adjuvant may strengthen the response, or permit memory cells to form. Examples are aluminium compounds, liposomes and even capsid proteins from *Neisseria meningitidis*.

Development

A lot of research goes into vaccine development each year, as many new vaccines are needed by the world, especially against malaria and HIV, and safer vaccines against TB are needed in the era of HIV infection. Influenza vaccines are different each year, due to changing circulating strains and new influenza antigens need to be determined for both northern and southern hemispheres.

Vaccine schedules

Countries often have a recommended list of vaccines that they advise their citizens to have. For some, it is compulsory for children to have certain vaccines at certain ages, although few countries do not respect the rights of the parents to decline vaccination. In many cases, vaccines are supplied free of charge by the government's health system (Fig. 3).

Post-exposure prophylaxis for viral infections

Introduction

The ideal form of prevention is avoidance of exposure to a pathogen. However, this is not always possible. Where there is no previous immunity, either by past infection or by vaccination, and the implications of a disease are severe, certain measures may be taken to prevent infection or to ameliorate clinical disease. Situations that warrant such attempts at prevention are usually where disease is potentially dangerous (Fig. 1), or where permanent damage or long-term implications are significant. Health-care workers are often exposed to pathogens infecting their patients, rape victims may be exposed to pathogens such as HIV and outbreaks in the community may expose a large number of people to a single pathogen. Prevention of infection of infants born to infected mothers also falls into this category.

Principle of post-exposure prophylaxis

Post-exposure prophylaxis (PEP) consists of several aspects – not just administration of treatment. Where possible, minimising the exposure should be attempted – for instance cleaning of wounds with soap or another suitable disinfectant. Continued exposure should be avoided – for instance, reallocation of a pregnant nurse to work where she is no longer exposed to children infected with varicella. Immunoprophylaxis may be of benefit, as vaccines can often be used to ameliorate or prevent clinical symptoms and immunoglobulin from immune individuals may assist when given to a non-immune individual.

Chemoprophylaxis refers to antiviral drugs given to limit or prevent the infection. Recommendations change from time to time, based on the availability of newer drugs, and the latest research. Readers should obtain the latest guidelines.

Viruses for which PEP is available

After exposure to many infections, normal human immunoglobulin has been used in the past, and for some, such as measles or polio, it can still be used.

Fig. 1 **Care needs to be taken when working with certain agents.** (Photo courtesy of CDC/Joel G. Breman.)

Table 1 **Viruses for which post-exposure prophylaxis (PEP) is available**

Virus	Scenarios (examples)
HIV	Needlestick (see Fig. 2) or splash injuries, rape, other sexual exposure, mother-to-child transmission
Hepatitis B	Needlestick/splash injuries, rape, other sexual exposure, mother-to-child transmission
Hepatitis A	Outbreaks in a crèche or community residence
Varicella	Mother-to-child transmission. immunocompromised persons
Herpes simplex	Mother-to-child transmission
Rabies	Bites from rabid animals
Viral haemorrhagic fevers	Exposure to public and health-care workers in an outbreak
Influenza	A potential pandemic situation

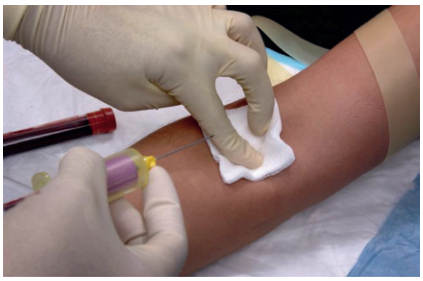


Fig. 2 **Taking blood needs to be done with care to avoid hazardous exposures.** (Photo courtesy of CDC/Jim Gathany.)

The most important viruses will be discussed here (see Table 1). It should be noted that different protocols may be available in different areas and may change from time to time, and so advice should always be obtained from someone familiar with management of PEP.

HIV

HIV prophylaxis should be started without delay after an exposure – preferably within an hour, but a delay, if even a few days, does not mean that prophylaxis should not be given. It is advisable to determine if the source of the exposure is infected with HIV, but if this is not possible, it should be assumed that exposure to HIV has occurred. Testing can sometimes delay initiation of prophylaxis – it is best to take the first dose of antiretroviral drugs as soon as possible and re-assess the need once testing has been completed. If the exposed person is HIV positive, post-exposure prophylaxis should not be given, as it will be of no use, and may limit future drug options for treatment.

The backbone of prophylaxis is similar to highly active antiretroviral therapy (HAART). Usually two nucleoside reverse transcriptase inhibitors (NRTIs) are used, with addition of another drug, usually a protease inhibitor (PI), at least for high-risk exposures.

High-risk exposures include the following:

- Deep percutaneous sharps injury
- Sharps visibly contaminated with blood
- Hollow needle from vein/artery
- Advanced HIV infection
- Viral load >100 000 c/ml
- Large volume splash
- Infants (usually high viral load).

In cases where transmission of drug-resistant HIV is suspected, drugs can be used for which no resistance is suspected – different NRTIs/PIs or the use of efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI). Nevirapine should be avoided in HIV-negative individuals. An expert should always be consulted.

In cases of mother-to-child prevention, the ideal treatment for the mother prior to delivery is HAART, to bring the viral load below detectable limits. The infant should also receive prophylaxis after birth.

Hepatitis B

If possible, the source of the exposure should be tested for hepatitis B infection. If in doubt, the case should be treated as if exposure occurred.

If the exposed person is known to be immune, no prophylaxis is necessary, as the antibody is very effective in preventing infection. A booster dose of vaccine can be considered.

If the exposed person is not immune, both antibody and vaccine should be administered as soon as possible. Two factors are important in the simultaneous administration of these – they should be administered in different locations, so as to avoid interference, and the vaccine should be administered into the deltoid muscle, as administration into a fatty area such as the gluteus may slow or limit the response. The immunoglobulin is given as a single dose at the time of exposure and the vaccine given as a series of three doses – at exposure, then 1 month later and 2 months after the second dose (this is an accelerated vaccination course – the third dose in a routine vaccination is at 5 months after the second dose). If the exposed person has previously been vaccinated, but antibody levels are low or absent although they were previously present, a booster dose should be given. In vaccine non-responders, immunoglobulin and a booster dose can be given.

Infants born to hepatitis B-infected mothers (Fig. 3) should also receive prophylaxis as soon as possible after birth. For both adult and infant prophylaxis, consult the latest guidelines to ensure that you are up to date.

Hepatitis A

The hepatitis A vaccine gives good protection even when given post-exposure, if the source of the exposure is identified in time. Where there has been a clearly defined exposure, but there has been a delay in identification of the risk and in taking action, normal human immunoglobulin (HNIG) can be given in addition to the vaccine. This is also recommended where there is a high risk of complications, e.g. co-existing liver disease. Vaccine should be given within 7 days of the index case's onset of symptoms, while HNIG can prevent disease if given up to 14 days after exposure, and can ameliorate disease even up to 28 days later, but is less effective in controlling community outbreaks, as, unlike the vaccine, it does not prevent viral excretion.

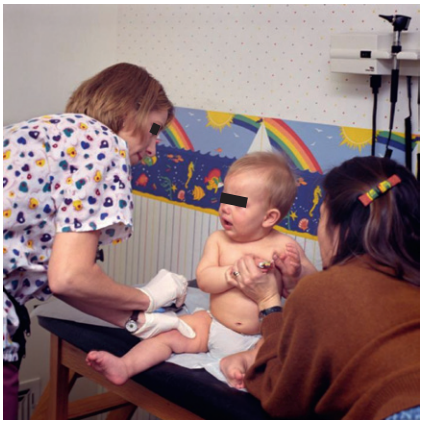


Fig. 3 **Baby getting a routine HBV vaccine.** (Photo courtesy of CDC/ Jim Gathany.)

Rabies

For details on risk assessment and the post-exposure prophylaxis regimen, see the chapter on Lyssa viruses and rabies. In brief, the protocol is as follows:

Wound care is essential – washing with soap or iodine or alcohol can help remove saliva or damage the infectious virus present. Stitching the wound is not advised, as this can assist viral entry.

Rabies vaccine is currently given according to the modified Essen schedule. Each dose is given into the deltoid muscle – not the gluteus muscle – except in infants, where the thigh is used. One should NOT wait for confirmation of disease in the animal before administration of prophylaxis!

Varicella

Varicella-zoster immunoglobulin (VZIG) can be given as PEP, and is recommended for individuals where there is a risk of severe varicella (e.g. pregnant women, immunocompromised individuals and neonates exposed in the first 7 days of life), significant exposure has occurred and no antibodies to varicella are present. The latter may take time to determine and needs to be weighed against the cost of VZIG when such decisions are made.

In neonates, acyclovir should be added if the mother has clinical varicella in the period of 4 days before delivery until 2 days after delivery.

Acyclovir given as prophylaxis should be started about 7–9 days after the start of the exposure period. If it is given before this time it is less effective, as it prevents initial viral replication, which is required to stimulate the immune response (this is an important reminder, as for many infections treatment and the immune system need to work together to be effective).

The live attenuated vaccine can be used as PEP, as cellular immune responses develop rapidly. The sooner it is given after exposure, the more effective it will be.

Herpes simplex

Primary herpes simplex infection during pregnancy is not always identified, but if an infection is identified caesarean section is recommended. Reactivation also poses a lesser risk to the infant both in utero and during labour. If identified in a case of vaginal delivery, treatment of both mother and infant with acyclovir can be given.

Adenoviruses

Classification (Table 1)

Table 1 **Classification of adenoviruses**

Family	Genera	Species	Serotypes
Adenoviridae	Mastadenovirus	A	12, 18, 31
		B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55
		C	1, 2, 5, 6
		D	8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 54
		E	4
		F	40, 41
		G	55

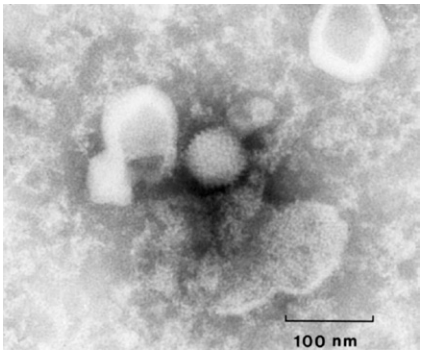


Fig. 2 **Electronmicroscopy of adenoviruses.** (Photo courtesy of Prof M Taylor, University of Pretoria.)

Pathogenesis

Adenoviral infection of target cells results in cell lysis and release of newly formed virions. An inflammatory cell infiltrate and the secretion of various cytokines accompany lytic infection. Infection may also be latent/persistent in the upper respiratory tract (tonsils and adenoids), gastrointestinal tract and lymphocytes, with periodic, asymptomatic shedding of virus in faecal and respiratory secretions.

Clinical picture

See Fig. 4.

Laboratory diagnosis

Faeces, throat swabs, nasopharyngeal aspirates, conjunctival swabs, urine, CSF, blood and tissue biopsies can be submitted to the laboratory for the diagnosis of adenovirus infection. Adenoviruses can be detected in respiratory secretions up to 1 week after the commencement of symptoms, in conjunctival specimens up to 2 weeks after the onset of ocular infection and in urine and stool samples from 2 weeks and up to 12 months after a respiratory or gastrointestinal infection.

Diagnosis may be attempted by means of direct and indirect methods.

Direct methods

- Isolation/culture

  Human embryonic kidney cells (HEK), hep–2, hela, and A549 cells are all suitable for the isolation of adenoviruses. Adenovirus CPE can be confirmed by the use of IIF, EIA or RIA.

  Adenoviruses 40/41 are fastidious and will only grow in a limited number of cell lines.

- Direct particle, antigen or genome detection

  Electron microscopy on faecal specimens is useful in the diagnosis of adeno 40/41 gastroenteritis but is not routinely available. A membrane

Structure (Figs 1 and 2)

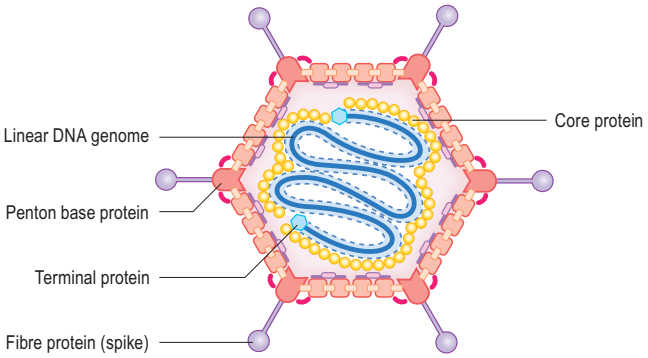


Fig. 1 **Adenovirus structure.**

Replication (Fig. 3)

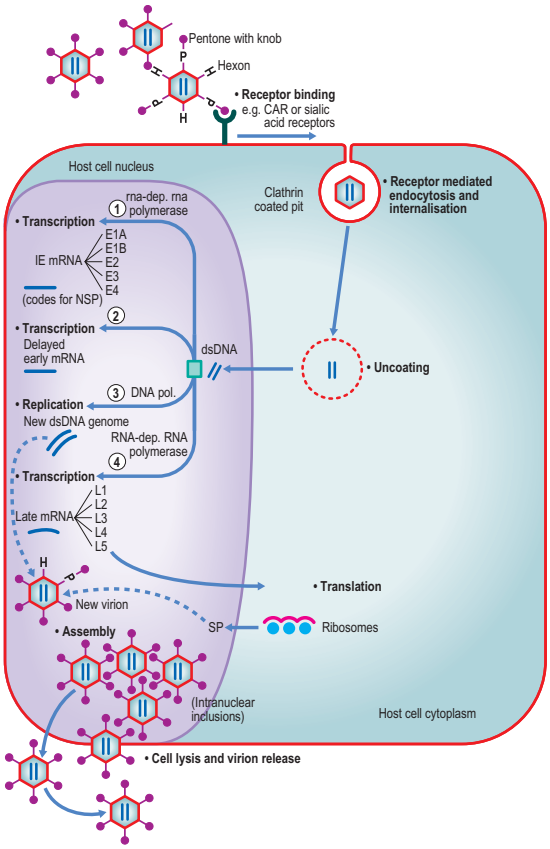


Fig. 3 **Adenovirus replication.**

Epidemiology (Table 2)

Table 2 **Epidemiology**

Clinical picture	Serotypes	Epidemiology
Respiratory disease		
■ URTI (coryza, pharyngitis, otitis media, tonsillitis)	1,2,3,5,7	Winter/spring
■ LRTI (croup, bronchiolitis, bronchitis, pneumonia)	3,7,4,21	Third most common cause of viral respiratory infection in children under the age of 4 years Transmitted via aerosols/direct contact
■ Acute respiratory disease in military recruits. (ARD)	3,4,7,14,21	Outbreaks Risk factors include: military recruits from various cultures in an overcrowded environment, early in their training programme, strenuous exercise and exertion Transmission via air filters in barracks has occurred Ad14 has been associated with a particularly severe and potentially fatal form of respiratory tract illness
Ocular disease		
■ Pharyngoconjunctival fever (PCF)	3,4,7,11-17, 19-21, 29	Outbreaks due to inadequate chlorination of swimming pools Transmission via swimming or swallowing of water
■ Epidemic keratoconjunctivitis (ECC)	8,19,37	Also known as 'Shipyard's eye' of Pearl Harbor fame Transmitted via direct contact, fomites, tonometry, instruments and solutions used in ophthalmology
Infantile gastroenteritis	40,41,12,31	Second most common cause of GE in children under the age of 2 years Faecal–oral transmission
Haemorrhagic cystitis	11,34,35	Immunocompromised patients

STOP THINK

- Experiments, using adenoviruses as viral **vectors** in gene therapy, vaccine research and cancer therapy are currently undertaken.
- Adenoviral vectors are investigated for their role in the treatment of cystic fibrosis (*CFTR* gene), muscle dystrophy (*dystrophin*), in anti-cancer treatment by altering expression of p53 and in prospective vaccines for HIV and rabies.

EIA is widely available and in use for the detection of antigens from adeno 40/41 from stool. The viral genome can be detected by molecular techniques including hybridisation techniques and PCR.

Indirect methods

- Serology – serological assays may yield false-negative results in immunocompromised patients and may only prove helpful in diagnosing infections retrospectively.

Only 20–50% of infected individuals mount specific IgM responses.

Prevention

No vaccine is currently available. Good infection control measures and adequate chlorination of swimming pools may prevent infections.

Treatment

Agents available for the treatment of adenoviral infections include ribavirin, cidofovir, gancyclovir and vidarabine. Immunotherapy, e.g. donor lymphocyte infusion and in vitro administration of cytotoxic T-cells may be considered in specific scenarios.

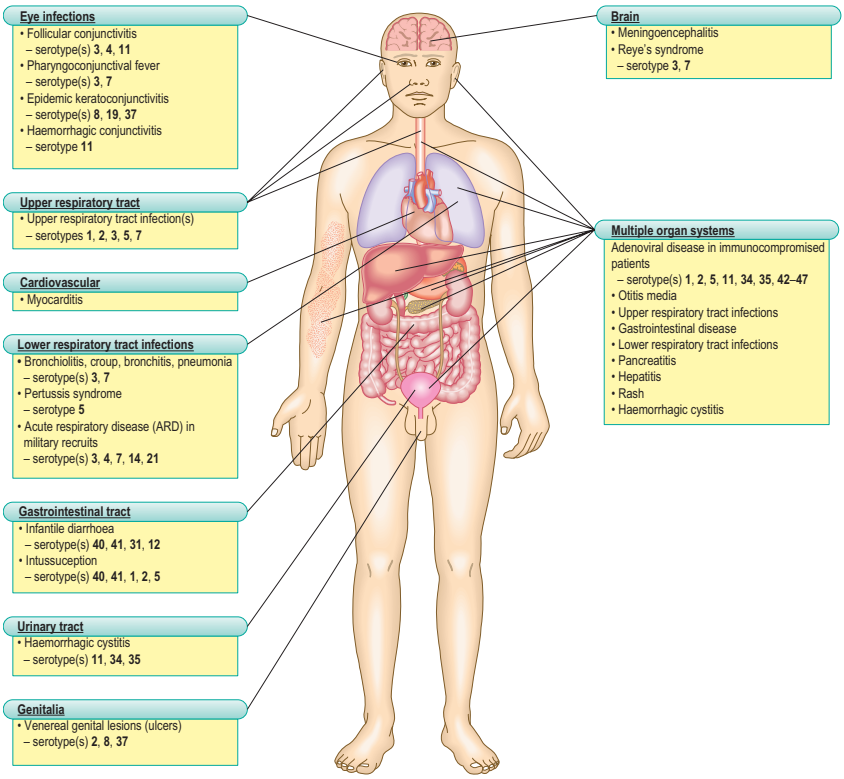


Fig. 4 **Adenoviruses – clinical picture.**

Herpes simplex and varicella zoster

Herpes viruses

Introduction

Historically membership of the family *Herpesviridae* has been based on the architecture of the virion. A typical virion consisting of a core, containing a linear double stranded DNA, an icosahedral capsid, a tegument and an envelope (Fig. 1A). More recently it has been found that they also share four distinct biological properties (Fig. 1B). Herpes viruses are widely found in nature, with currently eight reported herpesviruses which infected humans as their primary host and two zoonoses (Box 2).

Varicella zoster virus

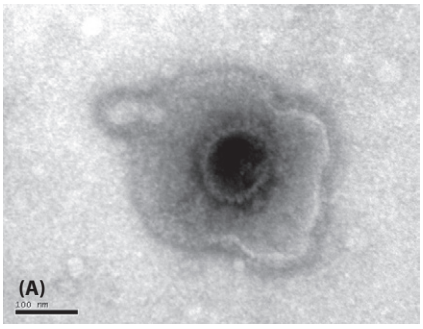
Varicella zoster virus (VZV) causes chickenpox during primary infection, when it infects and establishes latency in the cells of the dorsal column. Periodically, with increasing age or immunosuppression this virus may reactivate, causing shingles.

Epidemiology

Varicella zoster is a ubiquitous human pathogen with a global distribution. In developed countries chickenpox is typically a childhood illness, whilst in developing countries the majority of infections occur in young adults. The

Box 1 **Replication of herpesviruses**

1. They share a large array of enzymes involved in nucleic acid metabolism, DNA production and protein synthesis (although the exact enzymes may differ from one herpesvirus to another)
2. Synthesis of viral DNA and capsid assembly occurs in the nucleus; whilst final processing takes place in the cytoplasm
3. Production of infected progeny is invariably associated with lysis of the parent cell



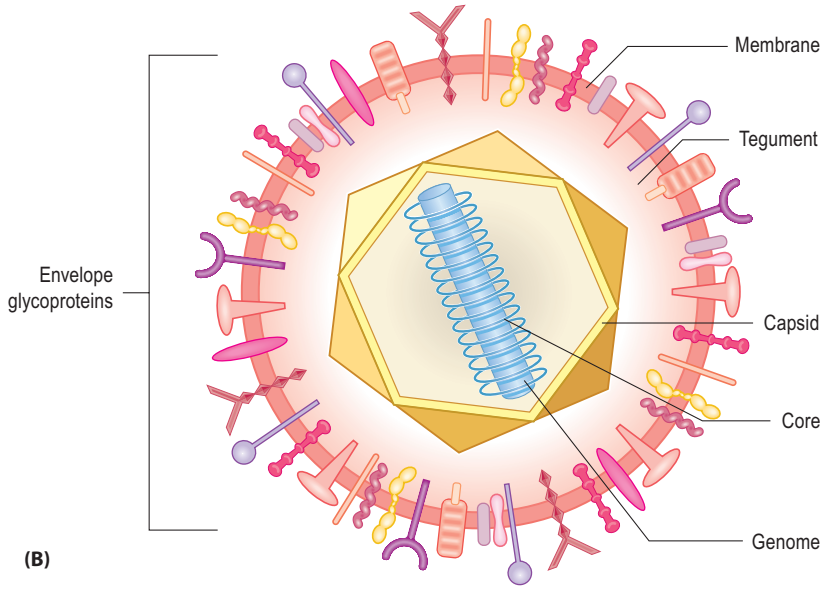


Fig. 1 **(A), Electron microscopy of a herpes virus.** (Photo courtesy of Dr David Hirst, Health Protection Agency, Bristol.) **(B),** Herpes virus showing structural features.

Box 2 **Herpesviruses of clinical significance**

Designation	Name	Abbreviation/Alternative name	Subfamily
Human herpesviruses (HHV)			
HHV-1	Herpes simplex virus 1	HSV-1	*Alphaherpesvirinae*
HHV-2	Herpes simplex virus 2	HSV-2	*Alphaherpesvirinae*
HHV-3	Varicella zoster virus	VZV	*Alphaherpesvirinae*
HHV-4	Cytomegalovirus	CMV	*Gammaherpesvirinae*
HHV-5	Epstein-Barr Virus	EBV	*Betaherpesvirinae*
HHV-6	Roseolovirus	HHV-6	*Betaherpesvirinae*
HHV-7	Roseolovirus	HHV-7	*Betaherpesvirinae*
HHV-8	Kaposi Sarcoma Herpes Virus	HHV-8/KSHV	*Gammaherpesvirinae*
Zoonotic herpesviruses			
CeHV-1	Cercopithecine herpesvirus-1	Herpesvirus B	*Alphaherpesvirinae*
MuHV-4	Murine gammaherpesvirus-68	MHV-68	*Gammaherpesviridae*

- How can varicella zoster infection be prevented?
- What problems do varicella and herpes simplex infections cause in the immunocompromised?

secondary attack rate (the number of new infections from a known case) is about 90%.

In the United Kingdom the incidence of VZV reactivation (shingles) is 3.4 cases per 1000 people per year. The incidence rises in those older than 60 years, such that the lifetime risk at 85 years is 50%. It also occurs in those who are immunosuppressed, particularly those who are HIV positive who have a 15 to 25 times increased risk of reactivation often affecting multiple dermatomes. Zoster is rare in children, except in those who acquired varicella during the first year of life or through intrauterine transmission.

Clinical

Chickenpox is transmitted via the respiratory route or by contact with infected fluid. Following an incubation phase of 10 to 21 days, a prodrome of fever, malaise, lethargy, irritability and anorexia is followed within 72 hours of becoming unwell by the appearance of an intensely pruritic vesicular rash. The rash has a classic distribution commencing first on the face, scalp and trunk then spreading to the limbs (centripedal distribution). Erythematous macules that evolve rapidly (in hours) form clear fluid-filled vesicles surrounded by erythema – so-called 'dew drop on a rose petal'. Lesions of mucous membranes including oropharynx, conjunctiva and vagina are common. New lesions emerge for 3 to 6 days with total number averaging about 300 lesions. After about 48 hours the lesions become turbid, umbilicate and crust. Vesicles develop in crops and therefore classically are at different stages of healing. Once lesions have crusted the individual is no longer infectious. Severe infection is associated with high level of exposure, e.g. household contacts, and in those with loss of skin integrity, e.g. eczema. Infection in the first 20 weeks of pregnancy carries a 2% risk of causing congenital varicella syndrome in the unborn child. Those babies born to mothers who develop chickenpox five days prior to or two days following delivery are most at risk of severe infection. Neonatal chickenpox carries a mortality rate of about 20%.

Complications from this disease (Box 3) are approximately 10 times more common in adults. Varicella pneumonia (Fig. 4) in healthy adults presented with fever, cough, tachypnoea, dyspnoea about 3 days after rash. Pregnant women, those with chronic lung disease and smokers are most at risk. Varicella gangrenosa is a necrotising fasciitis usually caused by *Streptococcus pyogenes* in those with chickenpox. Purpura fulminans is associated with arterial thrombosis and haemorrhagic gangrene. Interestingly, central nervous system complications have a bimodal distribution, being more common in those younger than 5 years and older than 20 years. Encephalitis may have a sudden onset with seizures and altered sensorium. Those with cerebellar disease have a slower onset with nystagmus, irritability, gait and speech disturbance. Encephalitis usually resolves within 24 to 72 hours, but ataxia may persist for longer.

VZV may reactivate from sensory ganglia to cause shingles. Most often patients present with vesicular eruption in one or multiple dermatomes of the face (trigeminal nerve) or the thoracic/lumbar area (Fig. 5). Reactivation without vesicles may also occur (*zoster sine herpete*). Multiple dermatomal involvement is associated with severe immunosuppression. Post-herpetic neuralgia (PHN) defined as dermatomal pain, which persists for more than 30 days. It is the most common complication of shingles and is reported in 9–19% of all zoster patients. The frequency of PHN is greater in older population groups and can be incapacitating causing a significant negative impact on quality of life.

Box 3 **Complications of varicella zoster infection**

Pneumonitis	Bacterial skin infection
Encephalitis	Arthritis
Nephritis	Pancreatitis
Thrombocytopenia	Orchitis
Pericarditis	Hepatitis

Diagnosis

The diagnosis of chickenpox and shingles is usually clinical. However, vesicular fluid can be tested using culture or increasingly by more sensitive molecular methods to confirm the diagnosis. These may be useful to differentiate from other virological diseases which can cause a vesicular rash including enteroviral infection, herpes simplex infection and smallpox (now only a potential bioweapon). Further testing may be useful where infection has occurred in vaccinated individuals or in the context of an outbreak. Historically a Tzanck smear, which is a smear of the ulcer base stained to show multinucleated giant cells, has been useful; however, it does not differentiate between herpetic causes of vesicular rash. Serology may be useful to determine whether someone has had chickenpox in the past, although history is often a more sensitive indicator.

Treatment

Chickenpox in healthy children is often not treated, unless there are other factors, e.g. immunosuppression; however, in adults where the risk of complications is much greater acyclovir or one of its derivatives is recommended. The sooner treatment is started the greater the benefit. Intravenous therapy is indicated in high-risk groups who have developed severe complications of infection, e.g. pneumonitis or encephalitis or neonatal chickenpox.

Treatment for shingles in those who are at risk of PHN should be started as early as possible in order to reduce the risk of severe sequelae.

Prevention

Vaccination, varicella zoster immunoglobulin (VZIG) and antivirals may all be used to prevent varicella infection. Vaccination is the most effective means of preventing this infection. A live attenuated vaccine has been available for almost 20 years and is part of the routine vaccination schedule of many developed countries. It is safe and effective, protecting 90–100% of children from severe disease. The most common side effect is a mild vesicular rash (average 5 spots) at the site of injection or at distant

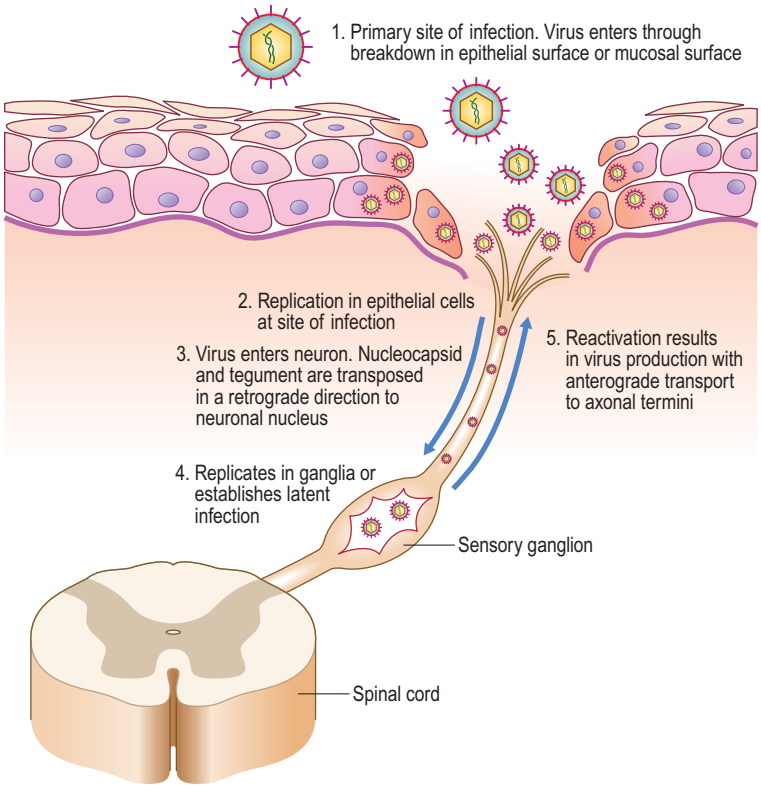


Fig. 2 **Primary infection, latency and reactivation of HSV.**

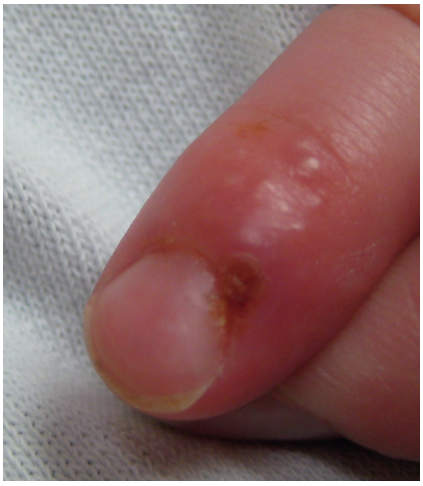


Fig. 3 **Herpetic whitlow.** (Photo courtesy of Prof James Heilman, University of Saskatchewan / University of British Columbia.)

sites which been observed in 2–4% of children and 5% of adults. As this is a live vaccine, it is contraindicated in pregnant women and in those who are severely immunocompromised. Post exposure prophylaxis with vaccination (within 72 hours of exposure) is safe and effective. Although not a licensed use, acyclovir given within 7 days of exposure can abort severe clinical disease and has been used when VZIG is not available. VZIG may be given to those who have significant exposure to infection, e.g. household contacts and those who are susceptible and at high risk of severe disease. For optimum benefit VZIG should be given within 72 hours (up to 96 hours) of exposure by intramuscular injection. Vaccination of older adults who have had chickenpox with a modified dose of varicella vaccine to prevent shingles is gaining acceptability.

Herpes simplex

There are two types of HSV – HSV-1, which preferentially infects the oral cavity, and HSV-2, which preferentially infects the genitalia. Both viruses can exist in either and other locations, e.g. nail bed causing herpetic whitlow. The virus remains latent after primary infection and may reactivate at any time (Fig. 2).

Epidemiology

These viruses are found globally. Only around 10% of infections are symptomatic. HSV-1 incidence starts to increase at a young age, whilst HSV-2 increases at puberty when sexual activity increases.

Clinical

The incubation period of HSV is around 5 days (2–12 days). HSV-1 causes a stomatitis in young children, affecting primarily the pharynx. Children may present with sore throat, drooling and fever. In adults the presentation tends to cause vesicular lesions on the buccal mucosa. This tends to be a mild disease. Herpes simplex is also responsible for causing severe disease in immunocompromised patients – disseminated herpes simplex infection characterised by a widespread vesicular rash, hepatitis and disseminated intravascular coagulation. HSV can also cause encephalitis classically affecting the parieto-temporal lobes, which may be devastating. Initially patients may present with confusion and progress to coma rapidly. The long-term recovery rate is poor. HSV-1 may cause overwhelming disease in the neonate – transmitted either during primary disease from the mother during delivery, or early in life through kissing or touch. Babies may present with localised disease or severe disseminated infection causing vesicular rash, hepatitis, apnoeic episodes, pneumonitis and thrombocytopenia.

HSV-2 causes genital ulceration. In women the primary site of disease is the cervix and vulva, whilst in men the lesions appear on the glans penis or prepuce; whilst in those engaging in anal sex lesions appear in the anus and rectum. Other sites may be affected depending on sexual practices. HSV-2 is more frequently associated with a meningitis rather than encephalitis.

Transmission

Transmission of infection is thorough close contact with infected lesions. This may occur through touch, e.g. sexual transmission, herpetic whitlow (Fig. 3), at delivery, and there is some evidence to support transmission through fomites.

Diagnosis

The diagnosis of HSV-related skin lesions is usually made clinically, although sometimes this can be difficult because of an atypical appearance. In these cases the diagnosis can be confirmed through culture or molecular testing. Samples should be taken as early as possible in the course of the illness when the viral load is highest and before crusting has occurred. The sensitivity of molecular methods is far superior to culture when the lesions are sampled late in disease. Molecular testing of cerebrospinal fluid is the test of choice for the diagnosis of HSV encephalitis.

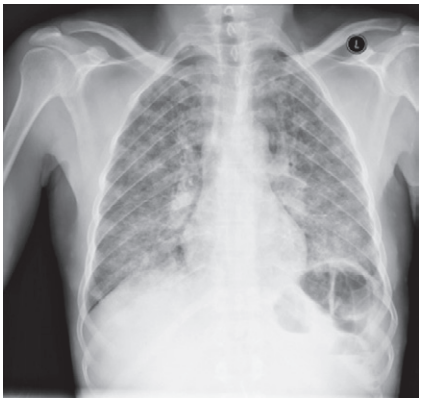


Fig. 4 **Chest X-ray showing severe chickenpox pneumonia.**

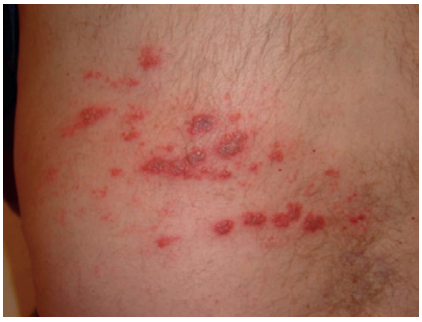


Fig. 5 **Varicella zoster in lumbar region.**

HSV serology is not a test that should be used to diagnose acute HSV infection, but may be useful in some specific acute clinical scenarios (Box 4). HSV serology is useful in a transplant setting to identify those with past HSV infection to inform clinicians about the use of prophylaxis against reactivation of HSV in the post-transplant period.

Box 4 **Uses of herpes serology**

1. Serodiscordant couples to inform transmission risk
2. Persistently negative genital ulceration of unknown cause
3. Herpes in pregnancy to determine mode of delivery

Treatment

Treatment of oral herpes is not always required and, although over-the-counter preparations of topical treatment are available, their efficacy is questionable. Topical treatment is not indicated for genital HSV infection. For primary infection guanosine analogues like aciclovir, famciclovir or valaciclovir may be used.

Reactivation may be prevented with low dose oral antiviral agent e.g. aciclovir. Conventionally the drug is taken for 6 months then stopped and recurrences monitored. Suppressive treatment may be restarted if recurrences are frequent and severe.

Prevention

HSV vaccine development is slow, but remains an important area of research.

Key points

- Varicella zoster virus and herpes simplex virus are DNA viruses belonging to the herpes family.
- These viruses can cause severe infections in the immunocompromised patient.
- These viruses can be treated with acyclovir and its derivatives.

Cytomegalovirus

Introduction

Cytomegalovirus (CMV) means 'large cell virus' and it refers to the swollen cells which contain large intranuclear inclusions that typify this viral infection (Fig. 1). Following primary infection, the virus can remain in a latent form in secretory glands, lymphoreticular tissue, kidney and other tissue and may reactivate at any time.

The virus

CMV, also known as human herpesvirus 5, belongs to the *Herpesviridae* family of viruses within the subfamily of *Betaherpesvirinae*, together with HHV-6 and HHV-7. Like all viruses in the *Herpesviridae* family, CMV is an enveloped, DNA virus.

Epidemiology

Seroepidemiological studies show CMV is universally distributed amongst human populations across the globe. Seroprevalence rates are near to 100% in lower socio-economic groups irrespective of country of origin and 50–60% in higher socio-economic groups in resource-rich countries. In general CMV is acquired early in life in resource-poor settings, whilst in resource-rich settings the prevalence of CMV infection is greater in select population groups, e.g. men who have sex with men and immigrant populations. CMV is the most frequent infectious cause of congenital disease globally, affecting 0.2–3% of live births in high income countries with higher rates reported from developing countries Chapter 50.

Transmission

CMV may be transmitted horizontally through contact with infected secretions or vertically from mother to unborn infant. Horizontal transmission of CMV may also occur through transfusion or organ transplantation, although screening procedures have reduced the risk of transmission via this route. Spread through airborne particles has not been documented. Following initial infection, virus may be present in urine, saliva, tears, semen and cervical secretions for years. CMV transmission rates are high where close contact with body fluids occurs, for example breastfeeding infants, in day centres, preschool age children and between sexual partners. Hospital workers do not appear to be at increased risk of infection when standard infection control procedures are in place.

Clinical features

Normal host

Acute CMV in the immunocompetent may cause an acute mononucleosis-like syndrome, which is generally a mild illness. The presentation of CMV mononucleosis is similar to that for Epstein-Barr virus (EBV). CMV is responsible for about 20–50% of heterophile negative mononucleosis and for 8% of all mononucleosis.

Congenital infection

Congenital CMV is characterised by a number of distinct clinical features (Table 1). The most concerning long-term problem is the effect on the brain and nervous system (see Chapter 50).

CMV is a potential cause of mortality in premature or low weight babies (less than 32 weeks or less than 1.5 kg). Breast milk is the most common route of infection with the advent of more stringent processes in place to prevent transfusion-associated infection. There is no consensus on the best form of management for these babies.

Immunocompromised

CMV in the immunocompromised host may be caused by primary infection, re-infection or reactivation. The severity of infection runs roughly parallel to severity of immune compromise. The most severe infections being seen in bone marrow transplant patients. In all transplant patients infection may be exogenous (from the donor tissue) or endogenous (due to reactivation in the recipient). The onset of infection usually occurs 4–8 weeks post-transplant, although with prophylaxis this may be delayed. In most centres patients are monitored weekly by CMV polymerase chain reaction (PCR) for evidence of increasing CMV viral load. Disseminated infection with colitis, retinitis (Fig. 2), pneumonitis and hepatitis may occur in these patients. Before the availability of antiretroviral therapy, CMV was a common cause of morbidity and mortality in patients with AIDS.

CMV infection in transplant patients may be prevented in a number of different ways. High-dose acyclovir may be used in the early post-transplant phase to prevent reactivation of any of the herpes viruses, prophylactic

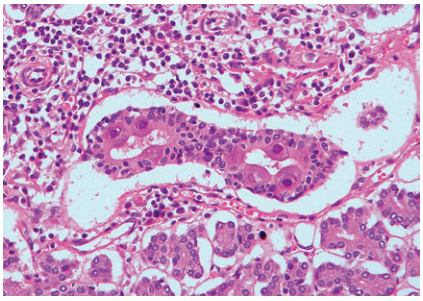


Fig. 1 **Haematoxylin and eosin stain of parotid gland showing inflammatory cells and inclusion bodies.** (Photo courtesy of Dr J Dempers, Stellenbosch University.)

Table 1 **Clinical features of congenital cytomegalovirus (CMV) infection**

Region affected	Abnormalities described
CNS	Microcephaly, mental retardation, spasticity, epilepsy, periventricular calcification
Eye	Choroiditis, optic atrophy
Ear	Sensorineural deafness (progressive)
Liver	Hepatosplenomegaly, hepatitis
Heart	Myocarditis
Blood	Thrombocytopenic purpura, haemolytic anaemia
Teeth	Enamel damage – brittle and yellow teeth

STOP THINK

- What other viral infections affect those patients who are immunocompromised?
- What are the ways in which viral infections can be prevented?

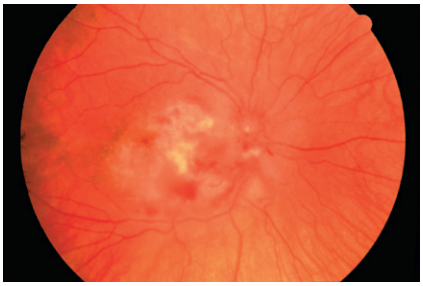


Fig. 2 **Cytomegalovirus (CMV) retinitis showing haemorrhage and exudates (so-called pizza pie).** (Photo courtesy of the National Eye Institute, National Institutes of Health, USA.)

doses of valganciclovir may be administered to patients for 100 days post-transplant or the CMV viral load may be closely monitored allowing early initiation of therapy.

Diagnosis

Molecular and viral culture

Molecular testing, most often realtime PCR are the mainstay of diagnosing infection in neonates and the immunocompromised. A positive urine sample taken in within the first 3 weeks of life is diagnostic of congenital CMV infection. Ethylenediaminetetraacetic acid (EDTA) plasma is used for the monitoring of CMV in the immunosuppressed. Other samples types, e.g. sputum, aqueous fluid or biopsy material may also be useful. Viral culture, although still available for diagnosis in some centres is now playing less of a role outside of its use in identifying drug resistance. CMV may be cultured from urines, saliva and the buffy coat of heparinised blood. CMV grows in fibroblasts and gives rise to foci of swollen multinucleated cells with characteristic intranuclear inclusions. Unlike herpes simplex virus (HSV), which may be evident in cell culture within 24 hours, CMV may take weeks to appear. More rapid methods of cell culture using monoclonal antibodies can reduce time to diagnosis (see Chapter 12).

Serology

Serological tests are the mainstay of the diagnosis of maternal infection. Seroconversion during pregnancy of maternal blood from CMV IgG negative to CMV IgG positive is a strong indicator of recent infection. The presence of CMV IgM antibody is associated with recent infection, although the positive predictive value of CMV IgM assays varies considerably. Avidity assays have improved the diagnosis of this condition, where low IgG avidity indicates recent infection and high IgG avidity past infection. Prenatal screening has not been shown to be cost effective. CMV excretion in pregnant women is not an adequate measure of infection, as those with past infection may shed periodically with no implications for the fetus.

Histology

In the age of molecular diagnostics, histology is less frequently used for CMV diagnosis. However, it may prove useful in cases where serum CMV viral load may be negative. It may also help differentiate a positive PCR result in those with disease caused by CMV and those in whom CMV has reactivated as an opportunist infection, but isn't the cause of the underlying condition.

Treatment

CMV lacks thymidine kinase and, therefore, acyclovir is not active against CMV, although it can be used for prophylaxis. Ganciclovir, which also acts as a chain terminator, is phosphorylated by a different enzyme and is used with some success. Intravitreal implants of ganciclovir have been used for the treatment of CMV retinitis. Valganciclovir is an orally active form of ganciclovir. Its use has revolutionised the prevention and treatment of CMV infection as it negates the risks associated with parenteral therapy. Cidofovir, a nucleotide analogue, is licensed for use in CMV retinitis. Foscarnet, which blocks the activity of viral DNA polymerases, has been used in the setting of ganciclovir resistance, but the serious renal toxic side effects limit its use.

Vaccination

There is currently no vaccine available for the prevention of CMV infection, but clinical trials are ongoing.

Key points

- Cytomegalovirus (CMV) is an important cause of congenital disease globally, those women with primary infection being most at risk of transmission to their unborn child.
- CMV may cause retinitis, pneumonitis, colitis and hepatitis in the immunocompromised patient.
- Ganciclovir, valganciclovir, cidofovir and foscarnet may be used for the treatment of CMV disease.

Epstein-Barr virus

Classification (Table 1)

Structure (see herpesvirus structure)

Replication (see herpesvirus replication, Chapter 23B).

Epidemiology

Epstein-Barr virus (EBV) infection is common amongst people living in developing countries. Children are subclinically infected early in life. In developed countries, infection generally occurs in early adulthood and infected individuals may present with infectious mononucleosis. Infection is mostly acquired by the oral route. Evidence of sexual transmission and transmission during blood transfusion and organ transplantation have been noted.

Pathogenesis (Fig. 1)

EBV infects and results in lysis of oropharyngeal epithelial cells, from where it may spread to passing B-cells in the associated lymphoid tissue. B-lymphocytes are infected and immortalised by the virus, resulting in a state of polyclonal activation. Most of the B-cells remain latently infected for life, while a small percentage may undergo a lytic infectious cycle. The infection is controlled by an intact cellular immune response where cytotoxic T-cells play a pivotal role. Memory B-cells are the main reservoirs for EBV reactivation and for the development of virus-related malignancies.

Viral gene expression differs between the lytic and latent stages of infection. Proteins expressed during the latent stage of infection include EBNAs (Epstein-Barr nuclear antigens) and LMPs (latent membrane proteins). These proteins aid in immortalising B cells and play a crucial role in oncogenesis. Lytic cycle proteins are divided into three classes based on the time at which they are produced with relation to DNA replication (immediate early, early and late proteins). Early proteins (EA) function as enzymes during replication. Late/structural proteins include viral capsid proteins (VCA) against which neutralising antibody responses are directed towards.

Clinical presentations

Infectious mononucleosis

After an incubation period of 30–50 days, patients develop a sore throat, fever, malaise, headache, rigors, anorexia, abdominal pain, neck swelling and neck stiffness. Cervical lymphadenopathy, hepatosplenomegaly and jaundice may be present on physical examination. Pharyngitis, with petechiae on the palate, and a grey-white membrane covering the tonsils are sometimes seen. Patients may present with a light morbilliform rash that usually only lasts for 24–48 hours. Neurological features are rare, but can include meningoencephalitis, transverse myelitis, Guillain-Barré syndrome and mono- or polyneuropathies. Pneumonia, pleural effusions, pericarditis and myocarditis may be present. Primary infection (infectious mononucleosis (IM)) resolves completely within 2 weeks, but relapses occur and intermittent fatigue may be experienced for the following 6–12 months. Complications of IM include airway obstruction due to oedema of oropharyngeal structures, secondary bacterial infection, liver necrosis, spontaneous splenic rupture, neurological sequelae and haematological/immunological phenomena.

B-cell lymphoproliferative disorders

Endemic Burkitt's lymphoma, Hodgkin's disease, X -linked lymphoproliferative disease or Duncan syndrome and post-transplant lymphoproliferative disease are all B-cell neoplasms where an association with EBV infection has been established.

Nasopharyngeal carcinoma

EBV is associated with the undifferentiated variant of squamous cell carcinoma of the nasopharynx, typically affecting males in the south of China.

Epstein-Barr virus infection in HIV/AIDS

Patients may present with Burkitt lymphoma in early HIV infection. Late stage infection/AIDS is associated with the development of primary CNS lymphoma or peripheral non-Hodgkin's lymphoma. Individuals may also present with Hodgkin's disease and primary effusion lymphoma,

Table 1 **Classification**

Herpesviridae		
Alphaherpesvirinae	***Betaherpesvirinae***	***Gammaherpesvirinae***
Herpes simplex virus 1 (HSV 1)	Cytomegalovirus (CMV)	Epstein Barr virus (EBV)
Herpes simplex virus 2 (HSV 2)	Human herpesvirus 6 (HHV 6)	Kaposi sarcoma associated herpesvirus/ human herpesvirus 8 (KSHV/HHV 8)
Herpes simplex virus 3 (VZV)	Human herpesvirus 7 (HHV 7)	

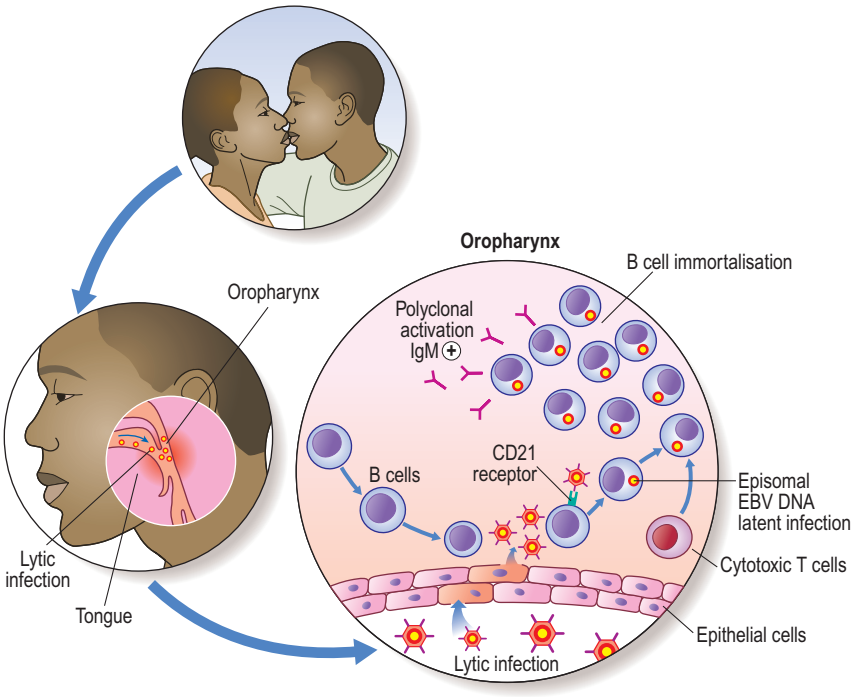
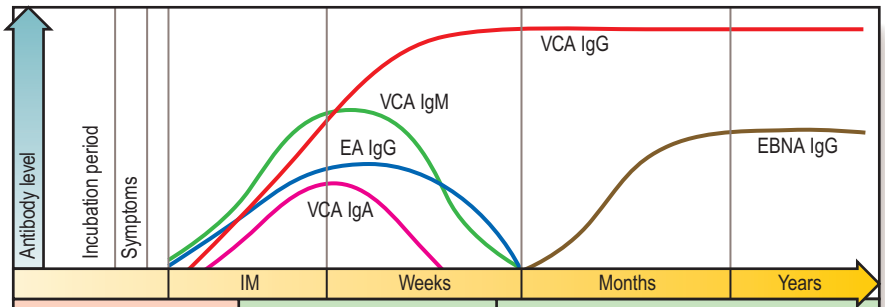


Fig. 1 **Epstein-Barr virus pathogenesis.**

EBV diagnostic test profile



Clinical entity	Non-specific tests		Antibodies against					
	Heterophylic antibodies	Atypical lymphocytes	VCA			EA		EBNA1
			IgM	IgG	IgA	EA(D)	EA(R)	IgG
No infection	–	–	–	–	–	–	–	–
Infectious mononucleosis	+++	+++	++	+++	+	+	(±)	–
Past infection	–	–	–	++	–	–	(±)	++
Reactivation	–	–	(+)	+++	++	++	+	(+)
Burkitt lymphoma	–	–	–	+++	–	(±)	++	+
Nasopharyngeal Ca	–	–	–	+++	++	+	(±)	++

Fig. 2 **Epstein Barr virus diagnostic test profile.**

where EBV is a cofactor to HHV-8. Oral hairy leukoplakia are painless, white, corrugated lesions on the lateral aspect of the tongue and indicative of active EBV replication.

Diagnosis

Non-specific tests

Tests for heterophylic IgM antibodies – These antibodies are typically present 1–2 weeks after acute EBV infection and have the ability to agglutinate sheep, horse and ox red blood cells. They can be measured by using the Paul Bunnell or Monospot tests, but false-negative values may be obtained in children under the age of four.

Atypical lymphocytes on peripheral blood smear – Patients with acute EBV infection experience a lymphocytosis, with atypical lymphocytes encompassing more than 10% of the total lymphocyte count. Atypical lymphocytes may also be present in acute infection with cytomegalovirus (CMV) and appear, like heterophylic antibodies, 1–2 weeks after the initial infection.

Specific tests

EBV culture – Special immortalised cell lines or co-culture systems can be used. This is usually regarded as labour intensive, costly and impractical in most diagnostic virology laboratories.

Specific serology (Fig. 2) – Antibodies against the lytic antigens, viral capsid antigen (VCA) and early antigen (EA) can be demonstrated from as early as the third week of infection. Antibodies against the latent antigen, EBNA, usually only appear 1 to 6 months after primary infection (in the convalescent phase) or as part of the latency profiles of the various malignancies associated with EBV infection.

Histology and immunohistochemistry – This can be performed on biopsies of various EBV-related malignancies.

Genetic studies – The BL *myc* translocation, t(8;14), t(2;8) or t(8;22) may be demonstrated.

Molecular techniques – EBV polymerase chain reaction (PCR) on the CSF is the method of choice for diagnosing EBV associated primary CNS lymphoma in AIDS patients.

Treatment

Infectious mononucleosis is treated symptomatically and may include analgesics for the sore throat and steroids for airway obstruction, neurological and haematological complications. BL is an aggressive tumour and is treated with chemotherapeutic regimes including cyclophosfamide. Nasopharyngeal carcinoma (NPC) carries a poor prognosis and is usually resistant to most chemotherapeutic agents. Oral hairy leukoplakia can be treated by acyclovir or one of its analogues. The timely instigation of HAART may protect against HIV-related opportunistic infections and malignancies.

Prevention

No vaccine against EBV is currently available.

Reduction of the dose of immunosuppressive regimes after transplantation may prevent the development of PTLD and HAART may be of benefit to HIV-positive patients.

Key points

- Epstein-Barr virus (EBV) infection is common amongst children in developing countries.
- It is transmitted via the oral route and termed 'kissing disease'.
- Primary EBV infection or infectious mononucleosis is the result of lytic replication of the virus in oropharyngeal epithelial cells.
- EBV immortalises B cells and induces a state of latency within them.
- EBV is an *oncovirus* and may give rise to various malignancies in immunosuppressed patients.
- EBV primary CNS lymphoma should be included in the differential diagnosis of an HIV/AIDS patient, presenting with a space-occupying lesion in the brain.
- Non-specific as well as specific diagnostic modalities can be used in diagnosing EBV infection.

Human herpesviruses 6, 7 and 8

HHV-6 and HHV-7

Classification (see herpesviruses)

Structure (see herpesvirus structure)

Replication (see herpesvirus replication, Chapter 23A)

Epidemiology

Infection with human herpesviruses 6 and 7 (HHV-6/HHV-7) commonly occurs in the age group 6–9 months, as well as in immunocompromised organ transplant recipients or patients suffering from HIV/AIDS. Infection with both viruses confers lifelong protective immunity to the host. The main route of transmission in babies is horizontally, via saliva, but both neonatal (breast milk) as well as congenital routes cannot be excluded. Acquisition of infection via blood transfusions or organ transplantation may occur in affected immunocompromised transplant recipients.

Pathogenesis

Both HHV-6 and HHV-7 cause lifelong infections and may be problematic for the host in both the case of primary infection as well as in reactivation from the latent state. The viruses are T-cell-tropic and neurotropic. Latency seems to occur in bone marrow progenitor cells, monocytes and macrophages. Infection results in direct and indirect T-cell lysis, as well as in a decrease in normal T-cell function. The viruses are masters at evading the immune system and employ many strategies, including up- and down regulation of cellular receptors, decrease in major histocompatibility complex (MHC-I) expression, abnormal cytokine regulation and decreased function and maturation of monocytes, macrophages and megakaryocytes in achieving this goal. Like other herpesviruses, viral gene expression includes immediate early, early and late transcripts.

Clinical picture

Primary infection with HHV-6 in infants is known as the sixth disease of childhood or *Roseola infantum* (exanthema subitum). A typical maculopapular rash (Fig. 1) involves the facial and trunk areas and develops

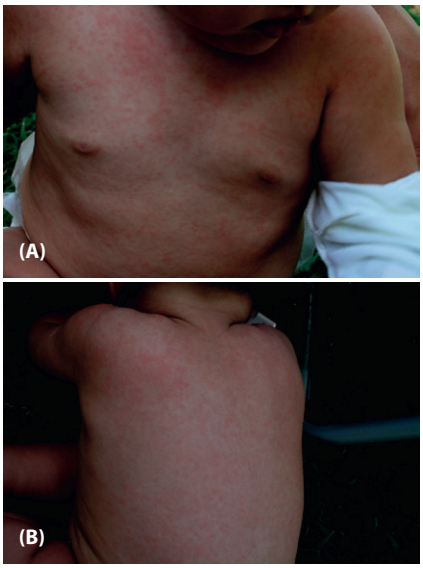


Fig. 1 **Roseola infantum on (A) the chest and (B) back of a baby.** (Photos courtesy of Emiliano Burzagli.)

3 to 4 days after the onset of fever. It may closely resemble measles or rubella infection. The rash of roseola may be accompanied by diarrhoea and transient leucopenia. Complications that may arise from the infection include febrile convulsions (in approximately 10% of affected infants), epilepsy, recurrent encephalitis, hepatitis, bone marrow suppression, gastrointestinal symptoms as well as respiratory complications like pneumonia and sinusitis.

Primary infection in adults is rare but may present with convulsions, encephalitis, flaccid paralysis and an infectious mononucleosis-like picture.

Reactivation of latent infection occurs in immunocompromised individuals and is the result of both the direct effects of viral replication, as well as indirect effects due to immune modulation. Solid organ transplant recipients (e.g. liver and kidney) may present with fever, rash, pneumonia, hepatitis, encephalitis, bone marrow suppression, graft rejection and reactivation of latent cytomegalovirus (CMV). Bone marrow or stem cell transplant recipients may present with decreased graft outgrowth, reactivation of latent CMV, encephalitis, diarrhoea and a skin rash, resembling that of graft-versus-host disease. Reactivation in these patients typically occurs 2 weeks to 1-month post-transplantation. Patients with AIDS and low CD4 counts are prone to HHV-6 and 7 reactivation, which may result in AIDS retinitis, pneumonitis, encephalitis, generalised lymphadenopathy and overall faster progression of HIV/AIDS. Reactivation of herpesviruses may however be prevented by the timely introduction of HAART.

HHV-6/7 have been implicated in other disease conditions affecting the heart, vascular and central nervous system (CNS). Possible associations include myocarditis, atherosclerosis, Alzheimer's disease and multiple sclerosis.

Laboratory diagnosis

Virus isolation from blood or CSF is impractical and requires the use of specialised lymphocyte culture lines. Available serological assays include IIF (indirect immunofluorescence), capture EIA, neutralization and immunoblot assays. Shortcomings of serology include the inability to distinguish between primary infection and reactivation as well as cross-reactions between HHV-6 and HHV-7. Molecular techniques, especially quantitative polymerase chain reaction (PCR) from blood, and CSF, can distinguish between latent infection and active primary infection or reactivation, and are fast becoming the mainstay of the diagnostic approach.

Treatment

Gancyclovir has proved useful in the treatment of HHV-6/7 *in vitro*.

Prevention

No vaccine to prevent infection with either HHV-6 or 7 is currently available.

Key points

- Infection with human herpesviruses (HHV) 6 and 7 occurs in the age group 6 to 9 months, as well as in immunocompromised organ transplant recipients or patients suffering from HIV/AIDS.
- The viruses give rise to lifelong, latent infections that may be reactivated in a state of immunosuppression.
- Primary infection with HHV-6 in infants is known as roseola infantum (exanthem a subitum).

HHV 8/Kaposi's sarcoma-associated herpesvirus (HHV-8/KSHV)

Classification (see herpesviruses)

Structure (see herpesvirus structure)

Replication (see herpesvirus replication, Chapter 23A).

Epidemiology

Routes of transmission of HHV-8/KSHV (Table 1)

Table 1 **Routes of transmission of HHV-8/KSHV**

Endemic areas, e.g. Africa, South America (children)	Non-endemic areas (adults)
■ Saliva (horizontal transmission)	■ Sexual ■ Parenteral (PBMC, plasma, serum, intravenous drug usage) ■ Solid organ transplant/bone marrow transplant ■ Transplacental, intrapartum

Types of Kaposi's sarcoma (Table 2)

Table 2 **Types of Kaposi's sarcoma**

Types of Kaposi sarcoma	Affected group(s)	Other features
■ Classical	Elderly men of eastern European or Mediterranean descent	Commonly affects only the skin with 10% chance of visceral involvement. Associated lymph oedema
■ Endemic	People of African descent	Association with lymphoma.
■ Post transplant	Immunocompromised transplant recipients (30 days after transplant)	Aggressive, 50% of cases have visceral involvement
■ Epidemic	AIDS patients (especially homo- and bisexual men)	Most common AIDS-related cancer in the USA

Pathogenesis

HHV-8/KSHV gives rise to both lytic as well as latent infections and expresses different genes in relation to these stages. It is an efficient *oncovirus* and is implicated in various malignancies, e.g. Kaposi's sarcoma (KS), body cavity/primary effusion lymphoma (PEL)) and multicentric Castleman's disease (MCD). Most infections with HHV-8/KSHV are asymptomatic, but a lapse in cell-mediated immunity may result in symptomatic primary or reactivated infection.

The endothelial derived spindle cells are the cells affected in KS and they predominantly express a latent antigen profile. PEL is also characterised by expression of a latent antigen profile, whereas lytic genes are predominantly expressed in MCD. Antibodies against HHV-8/KSHV and a high HHV-8 viral load are risk factors for the development of KS in infected individuals. HHV-8/KSHV exerts its oncogenic effects via several genes, primarily expressed during latency. Of these, LANA-1 (latency associated nuclear antigen) proves to be essential in replication of the latent genome, mitosis, regulation of transcription and inhibition of apoptosis. Other genes involved in transformation include *v-cyc* (a cyclin D variant), *vFlip* (an apoptosis inhibitor), *VEGF* and its receptor as well as viral interleukins 6 and 8. HHV-8/KSHV is also very efficient in evading components of both the innate as well as acquired immune systems.

Clinical picture

Moritz Kaposi, a Hungarian dermatologist, first described the characteristic red to purple coloured nodules of Kaposi's sarcoma in 1872. The skin of the extremities are usually involved, with particular affinity for the hands and feet (Fig. 2). Visceral involvement of the lungs and gastrointestinal tract may be present, especially in the case of post-transplant KS. KS developed 5–10 years after initial infection with the virus in AIDS patients prior to the introduction of HAART.

Primary effusion lymphoma (body cavity lymphoma) may present as malignant effusions of the peritoneal, pleural or pericardial spaces in AIDS patients.

MCD presents as a local lymphoproliferative disease of mediastinal, mesenteric or peripheral lymph nodes and commonly involves multiple sites.

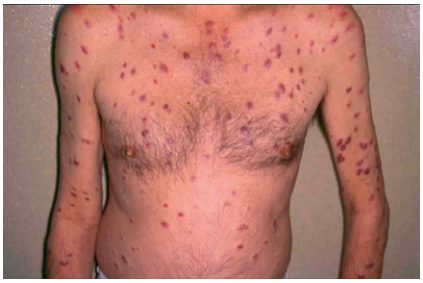


Fig. 2 **Kaposi's sarcoma on the trunk and upper extremities of a patient with AIDS.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

Laboratory diagnosis

Serological assays are widely available for the diagnosis of both latent and lytic infection.

Molecular assays, like quantitative PCR or viral load assays, have the additional benefit of monitoring immunosuppressed patients for disease progression or risk of developing disease. PCR can be performed on blood, saliva, semen and tissue specimens.

Culture of HHV-8/KSHV can be performed in specialised cell cultures, but this is generally regarded as impractical and not offered in routine diagnostic virology laboratories.

Treatment

Treatment modalities for KS include surgical excision, radiotherapy and various chemotherapeutic regimens. AZT, d4T, methotrexate and trimethoprim have also shown some benefit, but are currently not used as first-line agents in the treatment of KS. Intra-lesional interferon (that may be used in combination with AZT) and TNF (tumour necrosis factor) may be considered. With the introduction of HAART in 1996 came the promise of a new era in the management of KS and other HIV/AIDS related manifestations. HAART not only has a direct inhibitory effect on HHV-8/KSHV (especially in regimens containing a protease inhibitor), but also ensures the development of a stronger immune response to combat HHV-8. Classical anti-herpes agents, e.g. gancyclovir, cidofovir and foscarnet, only seem to aid in prevention of KS and not in treatment of established disease. The virus seems to be resistant to acyclovir.

Prevention

Early instigation of HAART in HIV positive patients and a decrease in the level of immunosuppression in transplant recipients may prevent disease and complications. No vaccine is currently available.

Key points

- HHV-8/KSHV is an efficient *oncovirus* and is the causative agent of various malignancies, such as Kaposi's sarcoma (KS), B-cell lymphomas, e.g. body cavity/primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD).
- Kaposi's sarcoma characterised by red to purple coloured nodules on the skin of the extremities, is the most common AIDS-related cancer in the USA.
- Early instigation of HAART in HIV positive patients may prevent HHV-8 disease.

Poxviruses

Smallpox (variola)

Epidemiology
Variola was confirmed as eradicated in 1979; the last naturally occurring case was seen in 1977, and the last laboratory-related case in 1978. Prior to that, it had been found worldwide. It was found only in humans.

Pathogenesis
Infection occurred via the respiratory route, and the infection became systemic as virus spread to the internal organs during the incubation period, and then to the skin.

Clinical picture
Fever with headache, backache, vomiting and constitutional symptoms developed after 10–14 days incubation. After a further 1–2 days a rash appeared, more severe on the extremities than on the trunk. It developed from a macular rash into vesicles by day 4–5, a pustule by day 7 (Fig. 3) and crusted by day 14. Lesions were seen on the hands, which differentiated it from chickenpox (Fig. 4). Ninety per cent of cases developed normal smallpox, with mortality ranging from <10% to 75%, depending on the extent of the rash, and the strain of smallpox, while 5% developed a modified form, less severe in nature. Five per cent developed flat smallpox, with slowly developing focal lesions, and a high mortality (~50%). Haemorrhagic smallpox was rare, but usually fatal, with bleeding into the skin and mucosae.

Vaccinia

Epidemiology
The precise origin of vaccinia is unknown. It has been widely used in the past as the vaccine for smallpox, reducing the mortality rate of smallpox to about 1%.

Pathogenesis
Usually inoculated deliberately into the skin, which is followed by local replication with limited systemic spread.

Clinical picture
Usually asymptomatic, but in some individuals potentially lethal complications occurred. About 8–10% experience a mild rash or local lesions. Contraindications, therefore, include conditions or treatment that may cause immunodeficiency, e.g. steroids, HIV infection; skin conditions, e.g. burns, dermatitis, eczema; pregnancy or breastfeeding. There is no absolute contraindication for vaccination in individuals exposed to smallpox.

Recombinant vaccinia virus
Due to its large genome, vaccinia is an ideal vector for insertion of foreign genes, particularly for use as vaccines. This allows the virus to produce the foreign proteins in its host, stimulating an immune response. Due to concern about safety, highly attenuated strains of vaccinia are used.

Monkeypox

Epidemiology
The natural hosts of monkeypox are squirrels and other rodents. Its natural distribution appears to be only Africa, although prairie dogs in the USA have been infected, probably due to exposure to imported pets. Humans and monkeys are occasional hosts.

Pathogenesis and clinical picture
Similar to smallpox, but less infectious, and with less severe symptoms, but a more severe lymphadenopathy, and a lower mortality rate.

Table 1 **Classification**

Family – Poxviridae	
Subfamily – Chordopoxvirinae	
Genus	**Species**
Orthopoxvirus	Variola virus Vaccinia virus Cowpox virus Monkeypox virus
Parapoxvirus	Orf virus
Molluscipoxvirus	Molluscum contagiosum virus
Yatapoxvirus	Yaba monkey tumour virus Tanapox virus

Table 2 **Structure**

- Large, complex viruses
- Brick-shaped viruses
- 200x400 nm
- Double-stranded DNA
- Membrane, capsid, two lateral bodies, surface tubules, a wide range of enzymes
- Replication occurs in the cytoplasm and is complex
- Only DNA virus to replicate fully in the cytoplasm

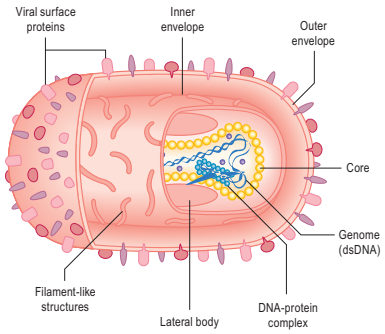


Fig. 1 **Structure of pox virus.**

Table 3 **Virological diagnosis**

Serology	**Isolation**	**Molecular**
Of no clinical use	Cell culture, chorioallantoic membrane of chicken embryos	Polymerase chain reaction – specialised centres

Note: the diagnosis is primarily on clinical grounds, with investigations done to determine the type of poxvirus involved if necessary – useful in cases of monkeypox, and important in suspected bioterrorism-related cases of smallpox. Currently only specialised institutions are able to investigate poxviruses

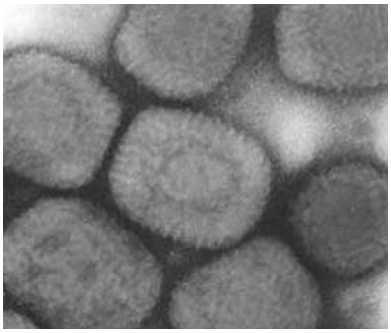


Fig. 2 **Molluscum contagiosum virus.** (Photo courtesy of CDC/ Hazelton PR, Gelderblom HR, Emerging Infectious Diseases journal.)

STOP THINK

- What other viral infections do we hope to eradicate?
- Which organisms are currently viewed as potential bioterrorism agents?

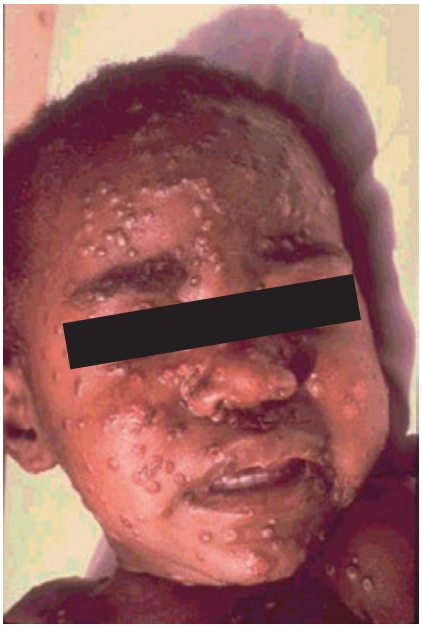


Fig. 3 **Smallpox lesions; see also Fig. 1 of Chapter 56.** (Photo courtesy of CDC/Cheryl Tyron.)

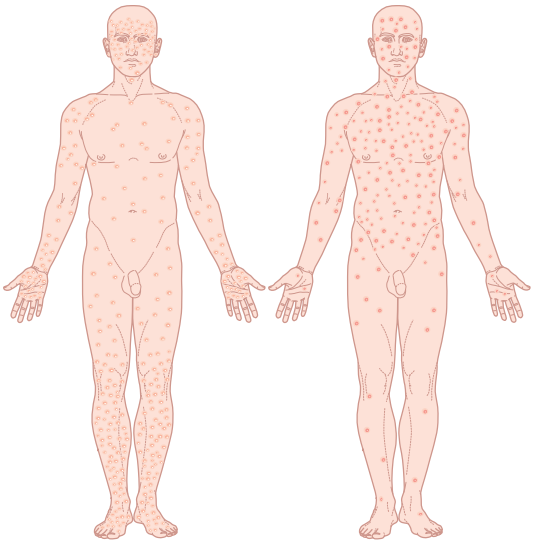


Fig. 4 **Difference between distribution of smallpox and chickenpox lesions.** (Modified from original diagram courtesy of CDC.)

Molluscum contagiosum

Epidemiology

Molluscum contagiosum appears worldwide. It is spread by cutaneous contact – sexually, and use of shared towels, as well as inoculation by scratching.

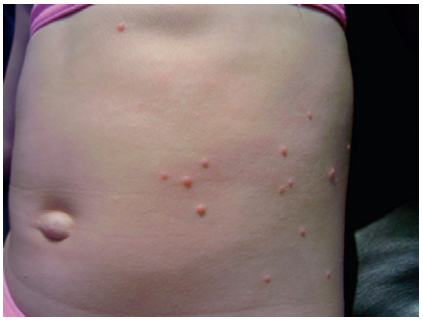


Fig. 5 **Molluscipox infection in a child with atopy. Infection was widespread, on the abdomen, buttocks and arms, spread by scratching.**

Pathogenesis

After incubation, a papule develops, which becomes a nodule within the epidermis. Inclusions are seen in the epidermal cells, enlarging with age. The basal membrane undergoes hyperplasia. The lesion is surrounded by a connective tissue capsule.

Clinical picture

Initially the lesion appears as a small papule. The mature lesion is a waxy or pearl-like lesion, often umbilicated. In immunocompetent individuals the disease is usually limited to 1–20 lesions, with spread often associated with areas of scratching. In individuals with impaired cell-mediated immunity, such as HIV infection, steroid use or atopy, widespread lesions may occur (Fig. 5) and the duration of the disease is longer.

Other poxviruses

Other poxviruses of rare clinical significance are Orf and Yatapoxviruses.

Specific treatment

No specific treatment is available for poxvirus infections. Most are self-limiting infections and do not require more than care of the lesion, and sometimes cosmetic removal in the case of molluscum. Monkeypox and smallpox infections have been ameliorated by the use of the smallpox vaccine. Cidofovir and vaccinia immune globulin (VIG) are being investigated for use as treatment.

Prevention

Vaccination with vaccinia was used in the past to prevent smallpox; its use is currently restricted to certain high-risk groups. It was used for pre- and post-exposure prophylaxis. It is also effective in protecting against cowpox and monkeypox infection. Isolation of cases is important and, in the laboratory, the use of specialised equipment provides protection.

Key points

- Smallpox was eradicated in the late 1970s and no longer occurs in the wild.
- Vaccinia is used as a vector in the design of various vaccines.

Polyomaviruses

Table 1 **Classification**
Family: Polyomaviridae
Genus: Polyomavirus
Species
BK virus
JC virus
SV 40 (vacuolising agent)
KI virus
WU virus
Merckel cell polyomavirus

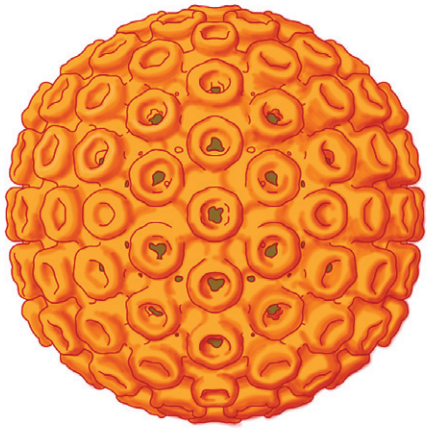


Fig. 1 **Structure of a polyomavirus.**

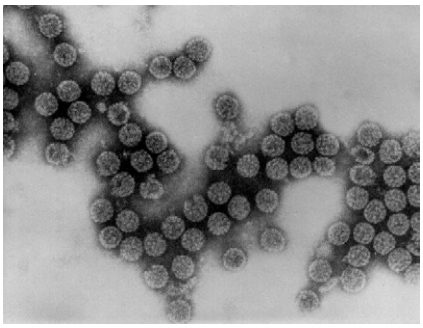


Fig. 2 **Electron micrograph of polyomaviruses.** (Photo courtesy of CDC/ Erskine Palmer.)

Replication

See Fig. 3.

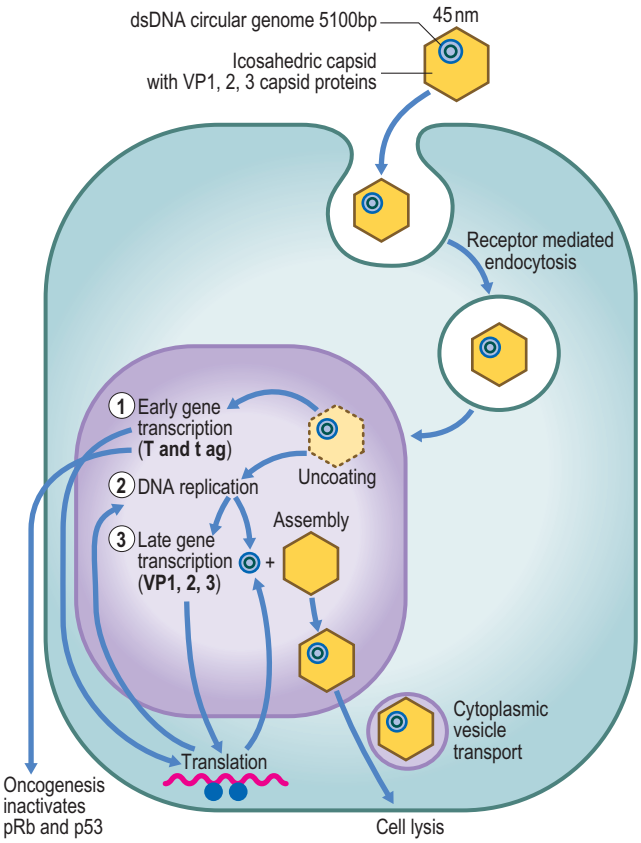


Fig. 3 **Replication cycle of polyomaviruses.**

Epidemiology

Polyomaviruses give rise to latent, lifelong infections, targeting both adult and paediatric immune competent populations. One hundred percent of children aged 10 years have been found to be seropositive for BK virus and approximately 80% of adults have antibodies against JC virus in certain surveyed populations. Infection is thought to be spread by the faecal-oral- and respiratory routes but congenital and sexual spread cannot be ruled out. Both BK and JC viruses are also excreted in urine. Infection in immune competent people is asymptomatic and generally not problematic. Immunocompromised patients, on the contrary, may experience reactivation of virus from latency and severe clinical manifestations due to lapses in cell mediated cytotoxic T-cell responses. KI and WU viruses are recently discovered polyomaviruses found in respiratory secretions and associated with respiratory tract infection. Merckel cell polyomavirus has been found in Merckel cell carcinomas as well as in respiratory secretions, but the significance is still uncertain.

Pathogenesis

The respiratory tract, gastrointestinal tracts and B-lymphocytes are thought to be the primary replication sites for polyomaviruses. A viraemia follows and results in infection of target organs, e.g. the central nervous system and urogenital tract. The viruses persist in the target organs as well as in B-lymphocytes, the spleen, lungs and tonsillar lymphoid tissue. Activation of persistent infection occurs as a result of immunosuppression and is seen in patients receiving bone marrow transplants, renal transplants and in patients suffering from HIV/AIDS. Pregnancy and advanced age may also reactivate latent virus.

Clinical picture

JC virus

Progressive multifocal leukoencephalopathy (PML)

PML presents as multiple, scattered, demyelinating, pin-sized lesions in the sub-cortical white matter of the brain on CT or MRI scan, as a result of

either primary infection or reactivation of JC virus. The lesions have irregular margins and show both clinical and histological evolution as the disease progresses. Histologically, the disease presents with brain atrophy and glioses and the oligodendrocytes are typically affected. The onset of disease is generally insidious and death occurs 4–6 months after the initial neurological presentation. PML has been described in patients with HIV/AIDS (especially in those with CD4 counts below 50 cells/ml) and malignancies, as well as in people receiving long-term suppressive therapy for autoimmune diseases such as SLE, rheumatoid arthritis, polymyositis, sarcoidosis and asthma. It may also present as part of the immune reconstitution syndrome (IRIS) in HIV-positive patients started on HAART. PML may present clinically with a mixed bag of neurological fallout, but speech, visual and motor disturbances are commonly found. It may also present with overt dementia.

Cancer

Both BK and JC viruses have been implicated in the development of malignancies in animals like hamsters and rodents. A possible association may exist between JC virus and colorectal cancer in humans, but it still needs to be proven.

BK virus

Urogenital infection

Both primary infection with BK virus in immunocompromised individuals and reactivation of persistent virus may result in disease of the urogenital tract. Renal transplant recipients may experience graft dysfunction, nephropathy, interstitial tubular nephritis and ureter stenosis. Haemorrhagic cystitis has been reported in 25% of bone marrow transplant recipients. BK viruria is also observed in organ transplant recipients, without evidence of symptomatic disease.

The significance of this finding is still uncertain.

Other infections

BK virus primary infection or reactivation may result in meningoencephalitis and interstitial pneumonitis in immunocompromised patients.

Other polyomaviruses

KI and WU viruses are believed to be involved in causing respiratory tract infections and are usually diagnosed by polymerase chain reaction (PCR). Merckel cell polyomavirus has been found in some Merckel cell carcinoma cells, but also in normal tissue. In the carcinoma cells, it is integrated into the host genome, has tumour-specific mutations and is no longer able to replicate.

Virological diagnosis

PML can be diagnosed by detection of JC virus nucleic acid in the CSF. Brain biopsies from suspicious lesions may be examined histologically and stained for JC Ag by using either immunofluorescence or immunoperoxidase stains. JC virus can be cultured by utilising primary human fetal glia cells, but this is considered impractical in most diagnostic virology laboratories.

Urine can be assessed by electronmicroscopy for intranuclear BK inclusions. Electronmicroscopy may also aid in distinguishing BK virus from cytomegalovirus (CMV), another virus commonly found in the urine of immunosuppressed patients.

Isolation of BK virus can be attempted on Vero or human embryonic kidney (HEK) cells. The presence of BK virus can also be established by the use of molecular techniques.

Serological techniques available for the polyomaviruses include haemagglutination inhibition, neutralising complement fixation and enzyme immunoassays. Intrathecal antibody synthesis and oligoclonal band detection can be performed on the CSF.

Treatment

PML may be treated with nucleoside analogues, e.g. ARA–A, ARA–C. The timely instigation of HAART may provide additional benefit to HIV/AIDS patients. Cidofovir may be used in combination with HAART to decrease viral replication. Immune modulatory agents, e.g. IFN alpha, IL 2 and heparansulphate may be potential treatment modalities in the future.

BK associated diseases may be treated by decreasing the level of immunosuppression in transplant recipients. Cidofovir, vidarabine and prostaglandin E2 may also be considered for therapy.

Prevention

No vaccine is currently available to prevent either JC or BK virus associated disease. Prevention of polyomavirus disease and decrease in the level of immunosuppression may be obtained by the early instigation of HAART in HIV-positive patients.

Key points

- Polyomaviruses are naked, double-stranded DNA viruses.
- They give rise to clinical disease in immunocompromised patients; especially transplant recipients and HIV/AIDS patients.
- JC virus is the cause of PML (progressive multifocal leucoencephalopathy), a demyelinating condition affecting the oligodendrocytes of the brain.
- Polyomavirus infection may present like multiple sclerosis and with various types of neurological fallout.
- BK virus is the cause of haemorrhagic cystitis in 25% of bone marrow transplant recipients.
- HAART may prevent the development of PML in HIV/AIDS patients.

Human papillomaviruses

Table 1 **Classification**
Family: Papillomaviridae
Genus: several, e.g. Alphapapillomavirus, Betapapillomavirus
Species:
Human papillomaviruses
82 genotypes assigned
100 fully sequenced genomes
possibly another 100 genotypes

Table 2 **Structure and replication**	
Structure	**Replication**
Icosahedral	**Target cells**
Non-enveloped Capsid: 72 capsomers Diameter: 52–53 nm Genome: ■ DNA ■ Double stranded ■ Circular ■ All genes transcribed from one strand ■ ~7800 bases	■ Mucosal and keratinised epithelium

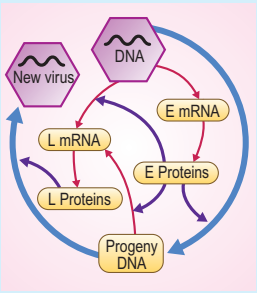


Fig. 1 **Replication of target cells.**

- Early proteins: E1–E7 (E1 and E2 stimulate replication and late-protein synthesis). E2 has a negative feedback on E6 and E7
- Late (structural) proteins L1, L2
- Late protein synthesis and capsid assembly only completed in differentiated epithelium
- L1: major capsid protein

Epidemiology

Different human papillomavirus (HPV) types infect squamous epithelium and skin. In a recent study where the foreheads of people with healthy normal skin was swabbed a prevalence of about 70% of HPV infection was detected. Using more sensitive techniques it may in future be found that asymptomatic skin infection is almost ubiquitous. Currently there are more than 100 fully sequenced genotypes and possibly another 100 genotypes not yet fully characterised. HPV1, 2, 3 and 4 cause common skin warts (Fig. 2 and see Fig. 3). HPV5 and 8 cause multiple warts and macules, and an increased risk of skin cancer in patients with epidermodysplasia verruciformis, a rare genetic immunodeficiency. The same types are associated with skin cancer in organ transplant patients. At least 30 genotypes infect the genital mucosa: HPV6 and 11 are associated with benign genital warts (Fig. 3), whereas HPV16 and 18 are most commonly found in cervical cancer. HPV16 is responsible for about 60% of cervical cancers and HPV18 for about 10% with other high-risk types, such as HPV45, 31, 33, 52 and 58, making a minor contribution. Genital HPV prevalence follows a U-shape curve in adult females with prevalence reaching a peak in women in their late teens and early twenties, due to sexual exposure, whereas after this it drops to start rising again after 50 years of age. The reason for the rise in the older group is the longer persistence of HPV in these persons, which increases the risk of cancer in this group. About 70% of women become infected with HPV during their lifetime. HPVs are also implicated in other genital cancers such as carcinoma of the vulva and penis. Less is known about the epidemiology of genital HPV in males. There is an association between HPV and oral cancers, and between HPV6 and 11 and juvenile laryngeal papillomatosis. Although these viruses are transmitted from mother to child during the birth process, children typically present with hoarseness, stridor or airway obstruction only at 2 to 3 years of age.

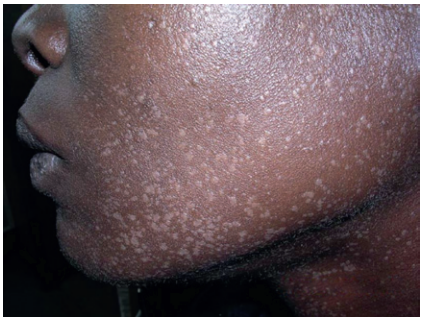


Fig. 2 **Verruca plana 'plane warts'.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

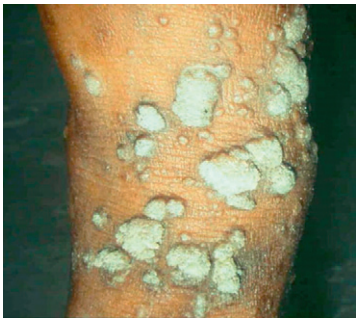


Fig. 3 **Verruca vulgaris (common warts).** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

Pathogenesis

HPV infects the basal layers of the epithelium through minor abrasions. In the basal cells HPV replicates the episomal genome at low copy numbers, with active viral replication and expression of structural genes (L1 and L2) only in differentiated epithelium, which helps HPVs to escape the immune response. HPV stimulates replication of epithelial cells even in the differentiated upper layers of epithelium. It, therefore, results in hyperplasia manifesting as white flat hyperplastic mucosal lesions or exophytic or endophytic warts on skin.

HPV is necessary for cervical oncogenesis, but other co-factors such as the use of oral contraceptives and smoking also play a role. The E6 and E7 proteins of highly oncogenic types HPV16 and 18 can immortalise cells in culture. E6 binds p53 preventing apoptosis after DNA damage and E7 binds pRb overriding the G1/S checkpoint which allows cell cycle progression and continuous replication. HPV16 and 18 are also associated with longer persistence of infection, which allows an increased chance of integration of HPV-DNA. During HPV-DNA integration E2 protein may be disrupted, allowing the uncontrolled expression of E6 and E7, which is a common finding in HPV-induced cervical cancer. Cervical

precancerous lesions usually arise in the transformation zone, an area of columnar epithelium at the cervical os which is under hormonal control and undergoes squamous metaplasia in adult females.

Clinical picture

Skin warts are typically found in children over 5 years old and in young adults. Skin warts usually regress within 2 years. Common warts (verruca vulgaris) are usually found on prominent regions subject to abrasion such as the fingers, hands and knees, they are raised (exophytic) and hard (hyperkeratotic). Plantar warts are deep, endophytic and painful. Flat warts are found on the hands and face, and have a flat and smooth surface.

Genital warts (condylomata accuminata) and bowenoid papules are found in the genital and anal region of young sexually active adults. Subclinical hyperplastic lesions on the penis, vulva or cervix can be detected under magnification by the use of 0.5% acetic acid.

The Papanicolaou (Pap) smear is used to detect pre-cancerous lesions of the cervix. These cytological smears are graded as low-grade or high-grade SIL (squamous intraepithelial lesion) depending on the morphology of the cells and the presence of koilocytes (HPV-infected squamous cells showing a characteristic cytopathic effect). Cytology (and in some settings also HPV-DNA testing) are used to determine whether somebody needs a cervical cone biopsy with histology, which is valuable not only as treatment for precancerous lesions but also is diagnostic for early cervical carcinoma. Cytology also correlates well with the degree of cervical intraepithelial neoplasia (CIN) on histological section, graded as CIN1–3 depending on whether the abnormal cells involve part or the full thickness of epithelium.

HPV can also infect the oral cavity causing focal epithelial hyperplasia. Juvenile laryngeal papillomatosis is a rare condition that follows infection of the trachea and larynx during the birth process. The larynx is most commonly involved but it may progress and spread distally to cause airway obstruction.

Virological diagnosis

The diagnosis of skin and genital warts is largely a clinical diagnosis but can be confirmed with histology. Molecular testing can identify and type the HPV involved. Cervical cytological screening by Pap smear is the mainstay of early detection of precancerous lesions. However, Pap smears have limited sensitivity and combining cervical cytology with HPV testing improves sensitivity and, therefore, is now part of the national recommendations in certain developed countries, such as the United States, for women older than 30 years. HPV testing also has special value in the discrimination of equivocal PAP smears results. Concurrent cytology and molecular testing is facilitated by the use of liquid-based cervical specimen collection. Methods that are available for the detection of HPV by molecular testing include: DNA-RNA hybrid capture, DNA probe hybridisation and polymerase chain reaction (PCR). The test currently most widely used is a DNA/RNA hybrid capture method which uses RNA probes to detect DNA and antibodies to capture these hybrids. The current method differentiates between high-risk and low-risk HPV types. Another method showing promise is realtime (quantitative) PCR. Genotyping HPV plays an important role in surveillance – especially with the advent of HPV vaccination.

A novel strategy for cervical screening that shows some promise is the use of vaginal fluid self-sampling with HPV testing.

Specific treatment

Warts usually respond to non-specific treatment such as freezing, curettage and podophyllin ointment. In severe cases of laryngeal papillomatosis specific treatment with intralesional interferon injection or local or systemic cidofovir can be used. Cidofovir should be used with caution since it is nephrotoxic.

The treatment of cervical precancerous lesions CIN2 or CIN3 is by procedures such as 'large loop excision of the transformation zone' (LLETZ). This procedure also provides tissue for histology which will guide the clinician in deciding whether a hysterectomy is indicated.

Prevention

Genital HPV is sexually transmitted and incidence can be reduced by limiting high-risk sexual exposures. Cervical cytological screening reduces the incidence of cervical cancer by 70% – this can be further improved by the combination with HPV testing. However, these strategies are expensive and frequent screening is unaffordable in the most developing countries. Recent clinical trials of a quadrivalent viral like particle (VLP) vaccine Gardasil® (Merck), consisting of empty L1 capsids of HPV16, 18, 6 and 11, produced in yeast cells, and a bivalent VLP HPV16 and 18 vaccine, Cervarix™ (GSK), produced in insect cells, have proven them highly effective in reducing type-specific HPV-infection and cervical precancerous lesions (CIN2 or greater). Gardasil® has the advantage of also preventing benign cervical warts and laryngeal papillomatosis in offspring. Due to the long pathogenesis of cervical carcinoma it will take many more years before the results of long-term studies would be able to show a reduction in cervical cancer in vaccine recipients; however, modelling predicts a very substantial reduction in cervical cancer. These vaccines may well prove to be the solution to cervical carcinoma prevention in industrialised and developing countries.

Human parvoviruses

Table 1 **Classification**

Family: Parvoviridae	Genus	Species
Subfamily: Parvovirinae	Erythrovirus	Human parvovirus B19
	Bocavirus	Human bocavirus
	Unclassified:	PARV4 and PARV5

Table 2 **Structure and replication**

Structure (see Fig. 1)

Non-enveloped
Capsid: 60 capsomers
Diameter: 18–26 nm
Genome:
- DNA
- single stranded
- linear
- positive or negative sense
- ~5500 bases

Empty capsids are seen

Replication (see Fig. 2)

Target cells
- Erythroid progenitor cells (parvovirus B19)
- Respiratory tract (bocavirus)

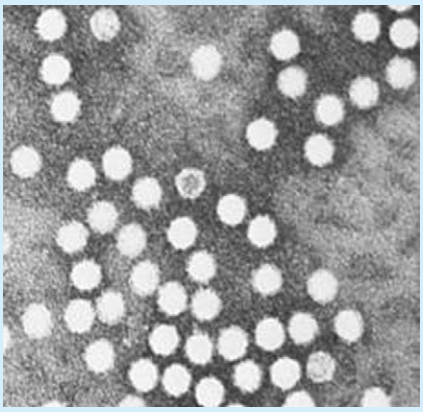


Fig. 1 **Parvoviruses.** (Photo courtesy of CDC.)

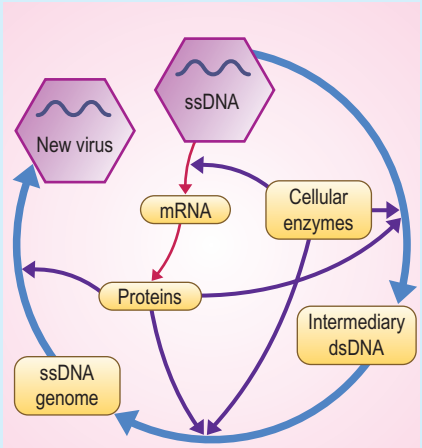


Fig. 2 **Replication cycle of parvoviruses.**

Genome and proteins

In Fig. 3 the parvovirus genome structure is laid out. NS is a non-structural protein that assists in replication. VP1 and VP2 are structural proteins, with VP1 differing from VP2 in length.

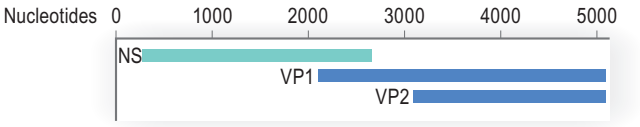


Fig. 3 **Parvovirus genome.**

Epidemiology

Human parvovirus B19

This virus infects only humans, and is found worldwide. Infection occurs throughout the year, while outbreaks tend to be more common between late winter and early summer, usually in schools, where children are infected – erythema infectiosum or fifth disease. Attack rates may be as high as 60%. Susceptible adults may be infected during contact with these outbreaks. By the age of 15, 50% have detectable IgG; this percentage rises with age. Infection occurs mostly through the respiratory tract, but can occur via transfusion of blood products.

Human bocavirus

This virus seems to be found worldwide and spreads via the respiratory route. It has been detected in 3–11% of cases of infantile lower respiratory tract disease. Winter appears to be the season with most infections, with infants under 2 years of age being the most at risk. Thus far, little else is known about its epidemiology.

Pathogenesis

Parvovirus B19

B19 infects cells using as a receptor the blood group P antigen, which is present on erythroid precursor cells, the main target cells, and also on endothelial cells and fetal myocardial cells, which may be involved in the pathogenesis of the rash, vasculitis, transplacental transmission and fetal cardiac involvement. Lytic infection of the erythroid precursors results in anaemia.

Entry is usually via the respiratory tract and the infection becomes systemic. It is normally brought under control by a neutralising antibody response. Immune complexes form, which result in the rash in erythema infectiosum as well as the arthropathy. Inadequate neutralising antibody production may result on a persisting infection.

Human bocavirus

Infants are infected via the respiratory tract, where it is believed to replicate and cause cytopathic effect. Little else is known about the pathogenesis.

STOP THINK

- What other viral infections are screened for on blood products?
- What factors would you consider as a differential diagnosis in a patient with chronic anaemia who develops an acute aplastic crisis?
- What other infections are important to look for in pregnancy?

Clinical picture

Human parvovirus B19

The incubation period for **parvovirus B19** ranges from 4 to 20 days, with most case-to-case intervals being between 6 and 11 days. Initially there is a mild fever, malaise, myalgia and headache, and after 2–5 days an erythema appears on the cheeks with circumoral pallor (Fig. 4), accompanied by a mild arthritis, which is more common in adults. 1–4 days later, a maculopapular rash appears bilaterally and symmetrically on the trunk and limbs, which becomes reticular or lace-like when it fades several days later (Fig. 5). In adults peripheral neuropathy, mainly in the fingers, and sometimes in the toes, is common. A reactive arthritis may occur, usually in adult women.

A transient aplastic crisis may occur in patients with haemolytic anaemia, and is usually serious. In pregnant women, fetal infection may result, with anaemia and myocarditis resulting in cardiac failure and severe oedema, known as hydrops fetalis. In the second trimester fetal loss is of concern. Congenital malformations are not believed to occur.

Human bocavirus

Human bocavirus presents as a respiratory tract infection in infants. See the chapter on respiratory virus infections.

Virological diagnosis

Serology: IgG indicates past infection, and usually persists for life.

IgM may persist up to 3 months after recent infection and does not cross the placenta.

Isolation: electron microscopic (EM) examination and culture are used in research settings.

Molecular: polymerase chain reaction (PCR) may detect virus in blood for weeks to months after infection, and is used to screen blood donations in some countries.

Human bocavirus is usually diagnosed by PCR.

Specific treatment

There is no specific antiviral treatment available for parvoviral infections. Symptomatic treatment for fever and arthropathy may be of value. Blood transfusions may be of benefit to fetuses and those with chronic anaemia. Chronic infections may respond to normal human immunoglobulin which contains neutralising antibodies.

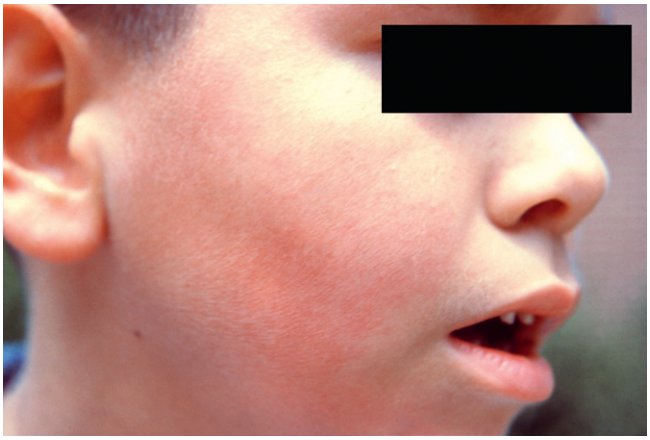


Fig. 4 **'Slapped cheek' rash of erythema infectiosum.** (Photo courtesy of CDC.)

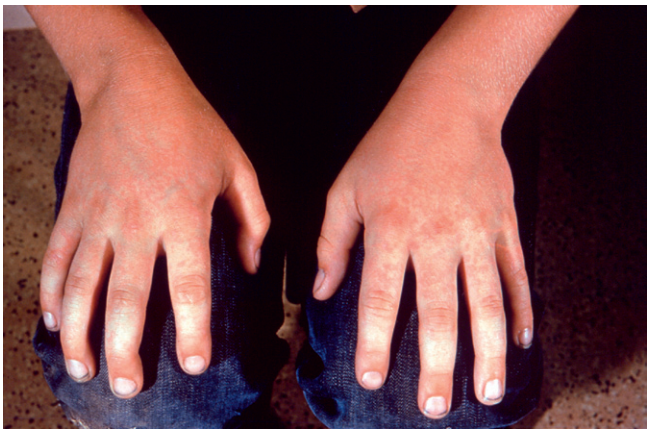


Fig. 5 **Erythema infectiosum. Note the reticulated rash on the hands.** (Photo courtesy of CDC.)

Prevention

Prevention is impractical as no vaccine exists, infections may be subclinical and viraemia is at its highest prior to the appearance of the specific rash. Persons known to have risk factors may be isolated from cases that are known and should be observed after exposure.

PARV4 and PARV5

PARV4 is a virus similar to parvovirus B19, and has been found in human blood. It can be spread by blood transfusion, trans-placentally, and possibly by needle sharing. It has been linked to anaemia and hydrops fetalis, but definite conclusions cannot be drawn at this point in time. It may be a harmless coincidental virus. PARV5 is related to PARV4, but even less is known about it. No diagnostic tools are available outside a pure research setting at present.

Key points

- Erythema infectiosum, or fifth disease, is a common childhood illness.
- Complications of parvovirus B19 infection can occur in patients with haemolytic anaemia, immune deficiencies or pregnancy.

Hepadnaviruses

Table 1 **Classification**

Family: Hepadnavirus	
Genus	***Species***
Hepadnaviridae	Orthohepadnavirus
Avihepadnavirus (birds)	Woodchuck hepatitis B virus
Orthohepadnavirus	Ground squirrel hepatitis B virus
	Human hepatitis B virus: 8 Genotypes A–H

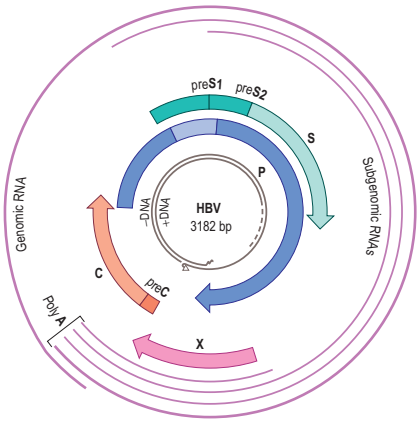


Fig. 1 **Genomic structure of hepatitis B virus.**

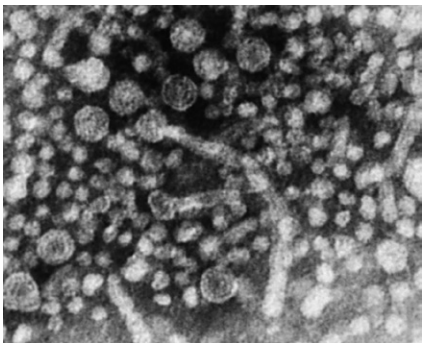


Fig. 2 **HBV electron micrograph showing spherical Dane particles (infectious virions).** (Photo courtesy of CDC/Betty Partin.)

Structure and replication

Hepatitis B virus (HBV) is a spherical, sometimes pleomorphic, virus with a diameter of 40–80 nanometers (Fig. 2). The genome is 3.2 kb of circular partially single-stranded DNA. Proteins are encoded by overlapping genes, from large to small: the polymerase, the envelope or surface proteins (pre-S2, pre-S1 and S), pre-core and core, and the X protein (Fig. 1).

Replication starts by attachment to receptors on the surface of hepatocytes. DNA is then transported to the nucleus where cellular polymerases extend the partially circular DNA to form a covalently closed circular DNA. mRNAs are then produced for each individual gene, which are translated to yield the respective proteins. A full genome mRNA is also transcribed which acts as template for reverse transcription and is thus needed for virus replication.

The polymerase gene encodes an enzyme with DNA polymerase and reverse transcriptase activity. The core proteins self-aggregate to form the nucleocapsid containing 240 capsomeres. When transcription starts upstream from the core in the pre-core region (from the full genome RNA) the e-antigen is produced, which is a soluble protein that does not form part of the virions but is secreted in the plasma. Production of e-antigen is thus normally coupled with viral transcription and is an indication of the viral load or amount of viruses produced except in cases where mutations in the pre-core or core region leads to stop codons. Pre-core or core mutant viruses are associated with high HBV loads despite the absence of e-antigen.

Three different envelope proteins are produced. If the mRNA includes only S, small (S) protein is made; if it includes S and pre-S1, a medium (M) protein is made and if it includes pre-S2, pre-S1 and S a large (L) protein is made. The envelope proteins are imbedded into a lipid membrane that is derived from the area between the endoplasmic reticulum and Golgi. During HBV infection S protein is overproduced, aggregating into filaments and vesicles that can be differentiated on electron microscopy (EM) from an infectious virus that contains a core.

As the core particles assemble, template RNAs are encapsulated and reverse transcription then proceeds within the nucleocapsid. Envelope proteins are embedded into the intracellular membranes where the core particles bud through the membranes to produce virions.

Epidemiology

Table 2

Route of infection	**Risk factors**
Blood and blood products	Intravenous drug use, needle stick injuries (health-care workers), haemophilia
Horizontal	Sexual transmission, Sub Saharan African children (rural areas)
Vertical	Especially when the mother is a highly infective HBeAg positive carrier (South East Asia)

Pathogenesis

Incubation period: 6–24 weeks (12–14 average).

Liver disease is caused by a combination of cytopathic viral effects and the immune response.

Acute hepatitis follows initial infection and is characterised by a high rate of viral replication. The damage to liver cells results in high blood concentration of liver enzymes and the obstruction of small bile tubes due to inflammation leads to the development of jaundice. In severe cases liver function may be so damaged that clotting is impaired or hepatic encephalopathy develops. This is referred to as acute fulminant hepatitis.

Between 1% and 4% of adults, about 70–80% of infants and children, and 90% of neonates will not clear the virus after acute infection and become chronically infected. Chronic HBV infection is defined as HBV infection lasting longer than 6 months. It includes a spectrum from chronic asymptomatic carrier state to chronic active hepatitis with rapid progression to cirrhosis.

Clinical picture

Acute HBV infection can be asymptomatic, cause acute febrile disease without jaundice or present as acute icteric hepatitis. Acute icteric hepatitis is characterised by loss of appetite, nausea, tiredness and fever. Jaundice develops later during the disease course. Excessive vomiting is a

bad prognostic sign. On biochemistry one finds elevated transaminases (often above 1000 IU) and mixed conjugated and unconjugated bilirubin elevation. Blood clotting is disturbed in severe cases as measured by increased clotting time. This can be accompanied with hepatic encephalopathy which is characterised by elevated plasma ammonia.

Virological diagnosis

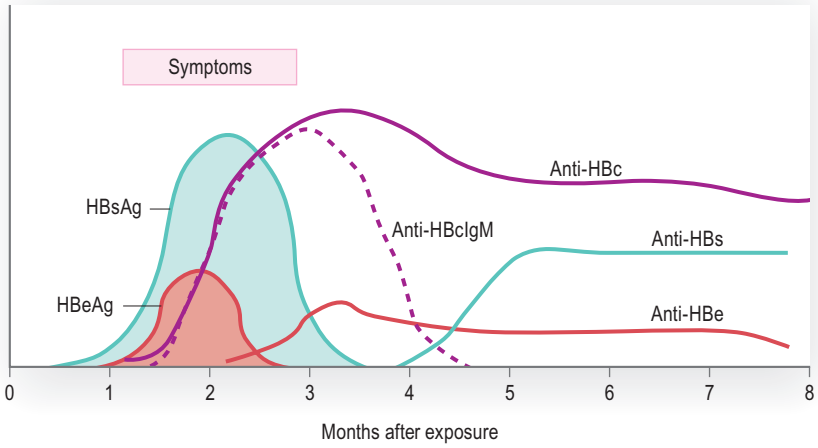


Fig. 3 **Acute hepatitis B infection with recovery.**

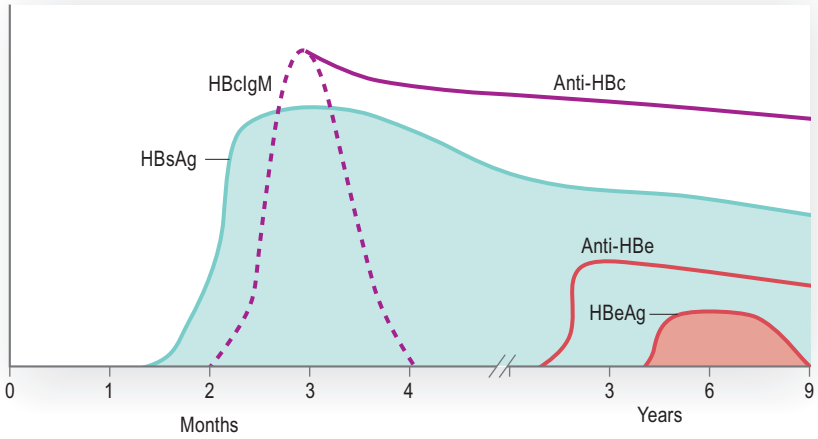


Fig. 4 **Chronic hepatitis B infection.**

HBV surface antigen (HBsAg) is a marker of active hepatitis. Since hepatitis B e-antigen (HBeAg) is normally associated with active viral replication, it is not only found in acute infections and patients with chronic active hepatitis, but also in a group of asymptomatic 'super carriers'. In patients with acute hepatitis B the disappearance of HBeAg and appearance of antibodies to eAg (anti-HBe) is indicative of recovery and in patients with chronic hepatitis B this is usually associated with therapy response or spontaneous recovery. Anti-HBV core IgM (anti-HBc IgM) is found during and shortly after acute hepatitis, when it is replaced by anti-core IgG (anti-HBc IgG). Anti-HBc IgM can also sometimes be seen in reactivation of chronic hepatitis. Anti-HBs is indicative of immunity: either recovery from acute infection or successful vaccination. In case of vaccination it would be the only marker present. In case of acute infection HBsAg usually disappears before anti-HBs can be detected Examples of typical temporal changes in serological markers in case of acute infection is shown in Fig. 3 and chronic infection in Fig. 4.

Specific treatment

Patients with chronic active hepatitis – characterised by the presence of HBeAg and/or high viral loads, liver cell damage as detected by elevated transaminases or necrosis and fibrosis on biopsy and who do not yet have cirrhosis – are likely to benefit from therapy. Interferon alpha or nucleoside analogues such as lamivudine, emtricitabine, adefovir dipivoxil, entecavir and tenofovir are available for treatment.

Prevention

Screening of blood products, vaccination of high-risk people or universal childhood immunisation in endemic countries as well as prevention of high-risk behaviours, such as intravenous drug use or unprotected sexual intercourse, are effective in preventing infection.

Non-immune individuals, who are exposed, such as health-care workers with needle stick injuries or children born to infected mothers, should receive post-exposure prophylaxis, which consists of simultaneous hepatitis B hyperimmunoglobulin and vaccine intramuscularly administered at different sites.

Retroviruses

In 1975 Howard Temin and David Baltimore received the Nobel prize for their discovery of the enzyme reverse transcriptase which overturned a basic principle of molecular biology – that genetic information only flows from DNA->RNA->protein. This enzyme is found, although not exclusively, in a group of viruses which belong to the family *Retroviridae*. This is a group of RNA viruses which replicate to produce DNA from RNA. The DNA is then incorporated into the host's genome by an enzyme called integrase. It is then known as a *provirus*. Thereafter, the virus is able to replicate as part of the hosts cell's DNA.

Endogenous retroviruses

In humans more than 98 000 human endogenous retroviruses (HERVs) have been identified. These viruses make up about 5% of the genome constituting over 30 lineages of related viruses. These viruses are spread through vertical transmission via the germline DNA. The role of HERVS in disease is not well understood. They may play a role in human cancers and autoimmune disease. While in baboons and mice there are ERVs that are transmissible as infectious viruses, this is not the case in humans. The focus will be on exogenous retroviruses affecting humans – namely HTLV and HIV (Table 1).

Classification of exogenous retroviruses

Table 1 **Classification**

Subfamily	Genus	Example	Host
Orthoretrovirinae	Alpharetrovirus	Rous sarcoma virus	Chickens
	Betaretrovirus	Mouse mammary tumour virus	Mice
	Gammaretrovirus	Murine leukaemia virus	Mice
	Deltaretrovirus	**Human T-lymphotropic virus**	**Humans**
	Epsilonretrovirus	Walleye dermal sarcoma virus	Fish
	Lentivirus	**Human immunodeficiency virus**	**Humans**
Spumaretrovirinae	Spumavirus	Primate foamy virus	Simians

Structure and replication

These enveloped viruses are about 100 nm in diameter (Fig. 1). They contain two identical single-stranded RNA molecules 7–10 kb in length. The envelope is made up of a protein capsid which is acquired from the host cell. The proteins include gag proteins, protease, pol and env proteins (Table 2). The replication cycle of retroviruses is shown in Fig. 2.

Human T-lymphotropic virus

Four distinct lineages of human T-lymphotropic virus (HTLV) have been identified since the discovery of Type 1 in the early 1980s. HTLV-1 is known to cause malignant and neurological disease in humans, whilst HTLV-2 has been associated with unusual cases of HTLV-1 associated myelopathy/tropic spastic paraparesis (HAM/TSP)-like illness. Little is known about HTLV-3 and 4 and

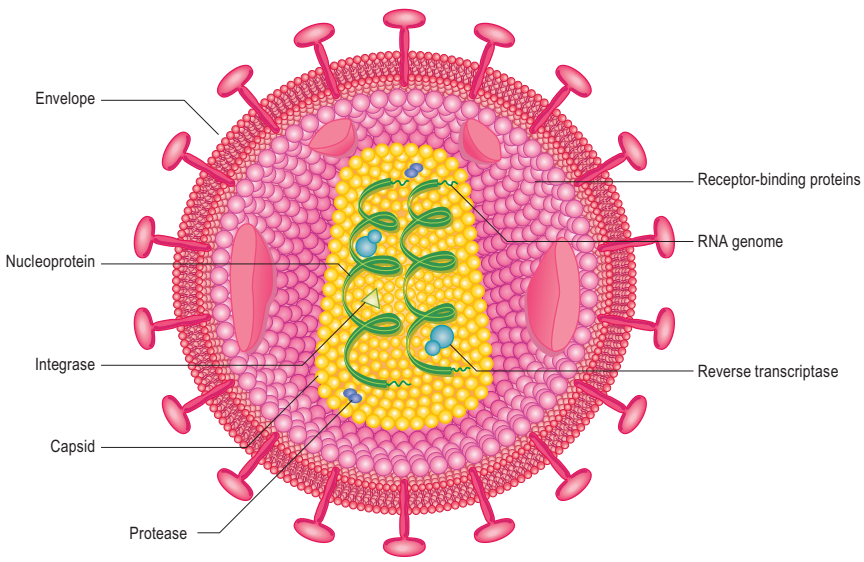


Fig. 1 **Structure of a retrovirus.**

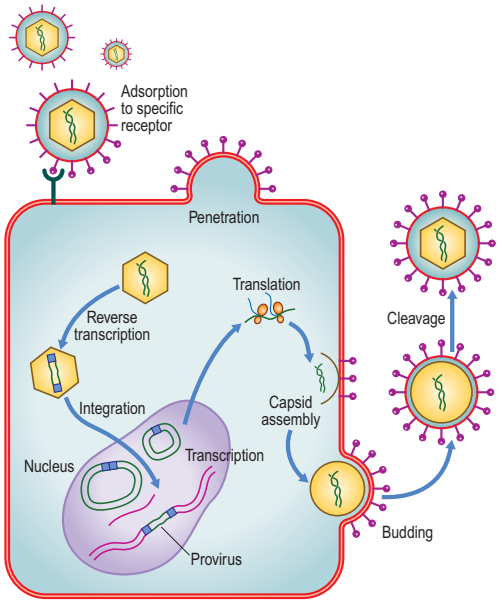


Fig. 2 **Lifecycle of a retrovirus.**

STOP THINK

- How do retroviruses replicate?
- What are the possible sites of drug action to block viral replications of retroviruses?

Table 2 **The main retroviral proteins and their function**

Protein	Function
Gag	Virus capsid
Protease	Maturation (differs in different retroviruses)
Pol	Synthesis of viral DNA and integration into host DNA
Env	Virus entry into the host cell

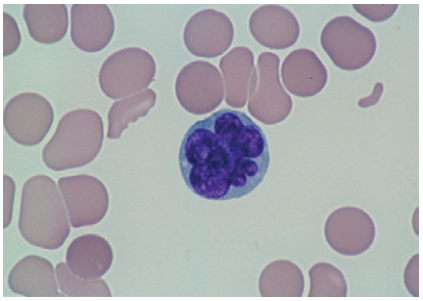


Fig. 3 **Flower cells owing to HTLV infection.** (Photo courtesy of Haematology, Tygerberg Hospital, University of Stellenbosch and National Health Laboratory Service.)

whether they cause disease in humans remains under discussion.

Epidemiology

An estimated 20 million people worldwide are infected with HTLV-1, most of these people will remain asymptomatic throughout life. The virus is endemic in Japan, the Caribbean, Papua New Guinea, Central and South America, Central Africa and in the aboriginal population in Australia. The seroprevalence differs between 0.1% and 30% amongst endemic populations and increases with age. It is higher among females. HTLV-1 can be transmitted sexually and via blood products containing cellular elements (HTLV-1 is predominantly cell associated). The risk of seroconverting after an infected transfusion is estimated at 40–60%. Transmission of HTLV-2 has been associated with sharing needles amongst intravenous drug users. HTLV-1 may also be transmitted vertically as result of prolonged (> 6 months) breastfeeding in high prevalence areas. The risk of an infected mother transmitting the virus to her baby may be as high as 30%.

Clinical picture

Adult T cell leukaemia/lymphoma

Adult T cell leukaemia/lymphoma (ATLL) (a non-Hodgkin's lymphoma) may present in a cutaneous form, as an acute T cell leukaemia, a chronic T cell leukaemia as well as in a smouldering variant form. The disease affects mainly males and the incubation period varies between 15 and 20 years. From 2% to 4% of HTLV-1 infections will result in the development of ATLL. Individuals with a high HTLV-1 viral load are more prone to developing the disease. Patients may present clinically with skin lesions, lymphadenopathy, hepatosplenomegaly and thirst. Biochemically, lactate dehydrogenase (LDH) as well as calcium levels may be raised. The malignant T cells have a characteristic polilobar or 'flower' appearance on peripheral blood or bone marrow smears (Fig. 3). The infected cells carry an immunophenotype of CD3+, CD4+, as well as CD25+ (Interleukin 2 receptor). HTLV-2 has been associated with lymphoproliferative disorder, but a causative association with this virus remains to be elucidated.

HTLV-1 associated myelopathy/tropic spastic paraparesis

HTLV-1 associated myelopathy/tropic spastic paraparesis (HAM/TSP) may occur shortly after initial infection with HTLV-1. The disease is more frequent in females than in males. HAM/TSP clinically presents with upper motor neuron signs. Symptoms may include chronic backache, faecal and urinary incontinence, constipation and a hyperactive bladder. Sensory disturbances may occur later in the course of the disease. HAM/TSP should be considered in the differential diagnosis of multiple sclerosis. The underlying pathology is that of multiple scattered atrophic and demyelinating lesions in both the cerebrum and spinal cord. Death usually occurs within 2 years from the onset of disease.

HTLV-1 is associated with a number of inflammatory diseases, e.g. uveitis, polymyositis, alveolitis, arthritis, thyroiditis, and Sjogrens disease. The exact role of the virus in many of these diseases remains unknown. HTLV-1 has also been associated with certain infections e.g. tuberculosis, crusted scabies and strongyloidiasis. HTLV-2 has also been associated with myelopathy.

Virological laboratory diagnosis

The laboratory diagnosis of HTLV infection is based on serological detection of antibody responses towards the virus and detection of the viral genome integrated (provirus) in the host CD4 cells.

Enzyme immunoassay (EIA) and latex agglutination assays are used as serological screening tests for HTLV infection and positive assays are then confirmed by means of a Western blot assay. Western blot assays are also able to distinguish between HTLV-1 and HTLV-2 infections. Polymerase chain reaction (PCR) may also be used for detecting viral nucleic acid in peripheral blood specimens from donors or in diagnosing HTLV infection in ATLL patients. In HAM/TSP, cerebrospinal fluid may be investigated for the presence of increased level of protein, lymphocytic pleocytosis, and HTLV-specific antibodies as well as detection of viral nucleic acid by PCR.

Treatment

ATLL generally has a very poor prognosis. The same treatment regimens as those that are used for Hodgkin lymphoma have been tried, but with varying success. Other tried therapies include bone marrow transplant, combination therapy with IFN alpha and AZT. Treatment options for HAM/TSP include symptomatic treatment, vitamin C, anabolic steroids, cytotoxic drugs, plasmaphoresis, IFN alpha and combinations of antiretroviral therapy.

Key points

- Retroviruses are RNA viruses that replicate via DNA using a reverse transcriptase enzyme.
- Both human T-lymphotropic virus (HTLV)-1 and 2 show a tropism for T lymphocytes and results in latent, lifelong infection of these cells.
- HTLV-1 is considered to be an oncovirus due to transactivation by 'tax'.
- HTLV-1 infection may lead to adult T cell leukaemia/lymphoma (ATLL) or HTLV-1 associated myelopathy/tropic spastic paraparesis (HAM/TSP).

Reoviruses causing human disease

Table 1 **Classification**
Family: Reoviridae
Genus
Rotavirus – gastroenteritis worldwide.
Coltivirus – 'Colorado tick fever virus' – febrile disease, meningitis and encephalitis; transmitted by ticks found in North America.
Seadornaviruses: Mosquito borne infections, South East Asia - flu-like disease and rarely encephalitis.
Orbiviruses: Many different species, outbreaks in non-human mammals (epizootics) transmitted by vectors–rarely infections of humans.
Rotavirus Species
Groups A to G (only A, B and C cause infections in humans)
Human rotaviruses classified into 11 G and 10 P serotypes have been identified
Most common human types: Group A (G types 1–4 and 9; P types 4 and 8)

Rotavirus

Structure and replication

A rotavirus (RV) has a 100 nm size non-enveloped trilaminar icosahedral nucleocapsid. The name is derived from the Latin *Rota*, which means wheel. On electron micrograph the viruses appear to have wheel-like spikes (Fig. 1).

The inner layer or core consists of VP1–VP3 and contains 11 double-stranded RNA segments. A second layer is made up of VP6 (group specific antigen) and third layer made up of VP7 (G or glycoprotein) and VP4 (P or protease sensitive protein). The segmented genome encodes structural (VP) and also five non-structural (NSP) proteins NSP1–NSP5.

Viruses attach to host cell sialic acid receptors on intestinal epithelium via VP4 on the outer layer; cleavage of VP4 by proteases such as trypsin into VP5 and VP8 enhances infectivity. Binding of integrin receptors is followed by receptor-mediated endocytosis. Inside the cytoplasm the VP7 layer disintegrates. Transcription and replication takes place inside the double-layered particle (DLP) and is mediated by VP1 (RNA-dependent polymerase) and VP3. Capped mRNAs are produced which are translated to proteins. The mRNAs also act as templates for the synthesis of negative strands which are transcribed to duplex RNA. This dsRNA is only created within the newly formed DLP which prevents dsRNA from eliciting a strong interferon response. DLP acquire VP4 and VP7 that forms the outer layer by budding through the endoplasmic reticulum, but shed the envelope membrane before final maturation. Mature virus particles are released by cell-lysis.

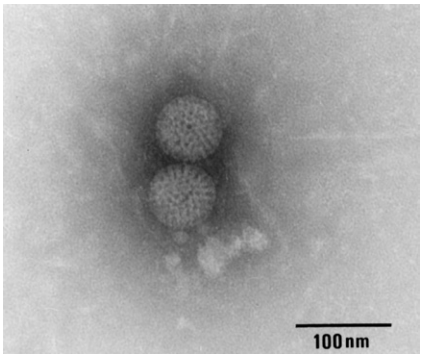


Fig. 1 **Stool specimen with rotaviruses.** (Photo courtesy of Prof M Taylor, University of Pretoria.)

Epidemiology

Rotaviruses are responsible for about 50% of infantile diarrhoea cases in the USA and are the leading cause of hospitalisation due to gastroenteritis. Worldwide, rotaviruses are responsible for 600 000 to 1 000 000 deaths per year. Although faecal-oral transmission is the most common route, respiratory infection is also possible. Primary infection usually occurs between 6 months and 2 years in temperate industrialised countries during the winter months although in Africa children are usually infected in the first year of life. The initial infection is usually the most severe. Outbreaks in neonatal intensive care units can cause severe morbidity.

Pathogenesis

Rotaviruses are spread mainly by faecal-oral transmission. Viruses are ingested and infect mature enterocytes. Rotavirus infection causes cytolysis and release of copious amounts of viral particles. Stool samples from children with rotavirus diarrhoea may contain more than 10^{10} to 10^{11} viruses per gram of faeces. The following mechanisms for rotavirus diarrhoea are postulated:

The cytopathic infection denudes the microvilli with atrophy of villi leading to a reduction in the absorption surface which is accompanied by the loss of brush border enzymes and transport mechanisms impairing digestion. Inflammatory cellular damage interferes with the absorption processes. A virus protein, NSP4, is also responsible for inducing secretory diarrhoea by acting as an enterotoxin and, finally, a release of enteric neurotransmitters results in increased motility and secretion of fluid.

The adaptive immune response to rotavirus follows presentation of rotavirus antigens in the gastrointestinal associated lymphoid tissue (GALT) to T-helper and B-cells. The innate immune response and cell-mediated response probably play an important role in viral clearance since IgA levels only peak after 14–17 days when the infection is usually already resolved. IgA titres tend to persist for the first year after infection. Type-specific antibodies towards VP4 and VP7 and group specific against VP6 are produced. Immunity is type-specific, although there is some degree of cross protection after the initial rotavirus infection. The first infection is therefore often the most severe. Rotavirus viraemia has been detected in some patients and may be responsible for systemic symptoms.

Clinical picture

The incubation period is 1–4 days. Onset of vomiting and diarrhoea is sudden and can rapidly lead to dehydration. The disease lasts from 3 to 9 days. Severity may vary from inapparent (more common if under 6 months of age or older than 5 years) to severe dehydration, especially in children with frequent vomiting that requires hospitalisation and intravenous rehydration. Up to 20 watery stools can be produced per day and a temperature of above 39°C is not an uncommon finding. Rotavirus is also associated with traveller's diarrhoea in adults, chronic diarrhoea in HIV-positive patients and disseminated disease in children with congenital immune deficits. Infants may also have respiratory and systemic disease.

Virological diagnosis

Since large quantities of virus are excreted in stools, direct detection methods such as electron microscopy, agglutination assays or enzyme immuno assays are relatively sensitive. Specialised techniques for serotyping rotaviruses also exist. Most human rotaviruses do not grow well in cell culture and cell culture of rotavirus is restricted to research laboratories. Electropherotyping, which separates the virus segments by gel electrophoresis, can also be used for virus typing. Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive technique for detecting the presence of rotavirus and the PCR products can be used to determine the particular virus genotype and strain.

Specific treatment

No specific treatment is available. In the acute phase of diarrhoea rehydration and electrolyte replacement either orally or parentally in children with severe vomiting or excessive dehydration is necessary. During the convalescent phase malabsorption and lactose intolerance may persist for a few days necessitating lactose-free or elemental feeding.

Prevention

Breastfeeding reduces the risk of serious diarrhoeal illness. General hygiene may reduce the risk of infection, but since the virus can also spread by aerosol it is not always effective. The first vaccine that became available was licensed in the USA in 1998 called Rotashield (a tetravalent vaccine consisting of human G1, G2 and G4 reassortant viruses on a G3 rhesus rotavirus backbone, also including the latter virus). However due to a 1:10 000 incidence of intussusception, the vaccine was withdrawn. A new vaccine called RotaTeq™ (Merck) was licensed in the USA at the start of 2006. This is a pentavalent human-bovine reassortant virus, which includes viruses with G1–G4 and p8 proteins. This vaccine was shown to be both effective in the prevention of rotavirus infection and safe. It is currently licensed in more than 85 countries, world-wide. Another vaccine, Rotarix® (GlaxoSmithKline), which is an attenuated human G1p8 strain has also been shown to be effective and safe, and is currently licensed in at least 120 countries, worldwide. Since there is such a big diversity of rotaviruses there is a concern that the use of these vaccines would lead to the selection of circulating virus strains that do not correspond to the vaccine viruses.

Coltivirus

Colorado tick fever virus, is a coltivirus, that is transmitted by the *Dermacentor andersoni* wood tick in Western North America. It causes a self-limiting flu-like disease in most patients. The illness is biphasic with an incubation period of 3–6 days, followed by improvement of symptoms after about 3 days, and a recurrence after 1–3 days. It rarely results in serious disease with meningitis, encephalitis, haemorrhagic fever, pericarditis and myocarditis. As no specific therapy is available, treatment is supportive and symptomatic.

Bunyaviruses

The family *Bunyaviridae* comprises numerous viruses assigned to four genera. They are about 80–120 nm in diameter and enveloped. The single-stranded negative-sense RNA genome is arranged in three segments, each contained in a separate nucleocapsid within the virion: L (large, coding for the viral transcriptase), M (medium, coding for the envelope glycoproteins), and S (small, coding for the nucleocapsid protein).

Each of the four genera within the family contains human-pathogenic species, plus many viruses not known to infect human beings (Table 1).

Most bunyaviruses are transmitted by biting insects, and some can also be transmitted through contact with tissues and blood of infected animals, e.g. during slaughter. An exception is the hantavirus genus: infection is acquired through inhalation, rarely inoculation, of rodent excreta. Infected rodents shed the virus, which remains infectious even when it dries out. If contaminated dust is inhaled, e.g. when sweeping out forest cabins, infection ensues.

During the Korean War in the 1950s, >3000 UN soldiers (Fig. 1) were affected by a febrile illness accompanied by renal failure termed haemorrhagic fever with renal syndrome (HFRS, Fig. 1). A similar disease entity had been described long ago in China and similar, mostly milder, illnesses from Russia and Scandinavia. In 1978 Lee et al. reported the isolation of a novel bunyavirus from the striped field mouse *Apodemus agrarius*. The new

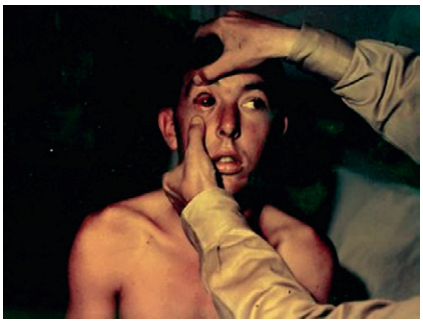


Fig. 1 **Patient suffering from haemorrhagic fever with renal syndrome (HFRS) during the Korean War.** (Photo courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, Washington, D.C.)

virus was named Hantaan after a river near the demilitarised zone dividing North and South Korea.

Clinically, HFRS begins acutely with fever >38.5°C accompanied by back and/or head and/or abdominal pain. This is followed by acute renal failure: proteinuria and/or haematuria, increased serum creatinine levels and oliguria. After the oliguric phase polyuria ensues followed by convalescence. However, mortality is considerable.

A similar but clinically less severe and seldom fatal form of HFRS occurs in parts of Europe, where it is known as Nephropathia epidemica (NE) and is caused by a different hantavirus carried by different rodent species.

In 1995, an apparently new, severe respiratory disease occurred in the 'Four Corners' region (where the states of Arizona, Utah, Colorado and New Mexico meet) of the United States of America. It is characterised by fever, myalgia, nausea and vomiting, cough, tachypnoea, tachycardia, hypotension and shortness of breath late in the course of disease. It turned out to be caused by a hitherto unknown species of hantavirus, the first of a long list of hantaviruses now known to occur throughout the New World. They are carried by different species of rodents and affect not primarily the kidneys but the lung, causing hantavirus (cardio)pulmonary syndrome (HPS, HCPS). The first of these viruses is now known as sin nombre virus (Spanish for 'without name'), seeing that local tourism stakeholders had objected to a name reflecting the locality of its first isolation, Four Corners or Muerto Cañon, fearing for the region's reputation.

With the exception of Andes virus, one of the South American hantaviruses causing HPS, hantaviruses are not transmitted from person to person.

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World Health Organization (WHO): Fact sheet N°207: Rift Valley fever (revised May 2010). http://www.who.int/mediacentre/factsheets/fs207/en/index.html

Table 1 **Classification**

Genus	Important species infecting humans	Transmitted through
Orthobunyavirus	California encephalitis Tahyna, Oropouche viruses	Mosquitoes
Phlebovirus	Sandfly fever (also known as phlebotomus or pappataci fever), Naples, Toscana, Sicilia viruses	Phlebotomid sandfly
	Rift Valley fever virus (see map, Fig. 2)	Mosquitoes; contact with infected animal blood and tissues
Nairovirus	Crimean–Congo haemorrhagic fever (CCHF) virus (see map, Fig. 2)	Ixodid ticks Contact with infected animal blood and tissues Nosocomially from infected human patient
Hantavirus	Hantaan, Dobrava-Belgrade, Seoul, Puumala, Sin Nombre, Sangassou viruses (see map, Fig. 2 and Fig. 3)	Rodent excreta and tissue via inhalation or inoculation (Figs. 3 and 4)

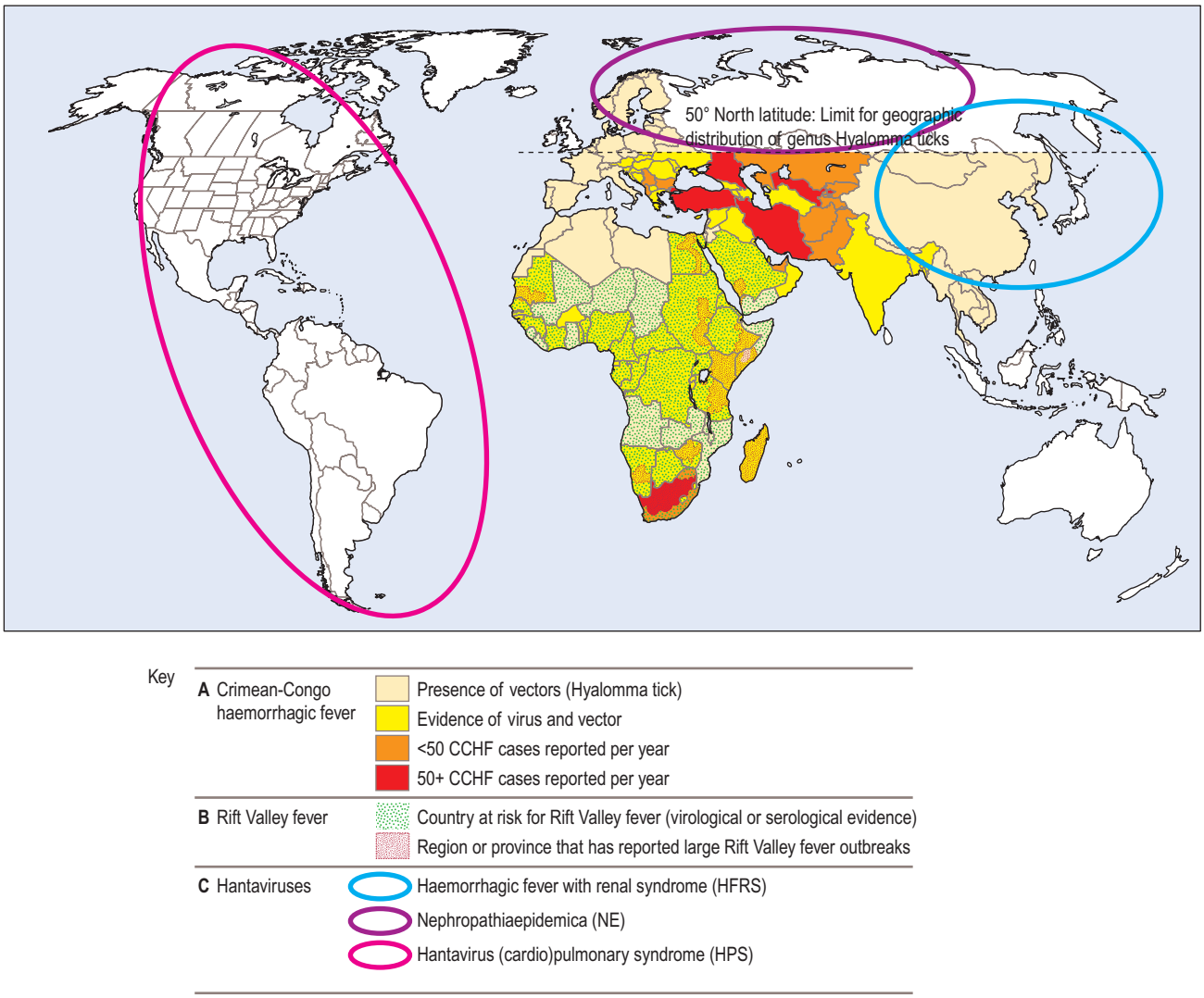

Fig. 2 **Geographic distribution of important diseases caused by bunyaviruses: a) Crimean-Congo haemorrhagic fever, b) Rift Valley fever, and c) Hantaviruses causing haemorrhagic fever with renal syndrome (HFRS), nephropathiaepidemica (NE) and hantavirus (cardio)pulmonary syndrome (HPS)** (Data from WHO, Johnson et al.)

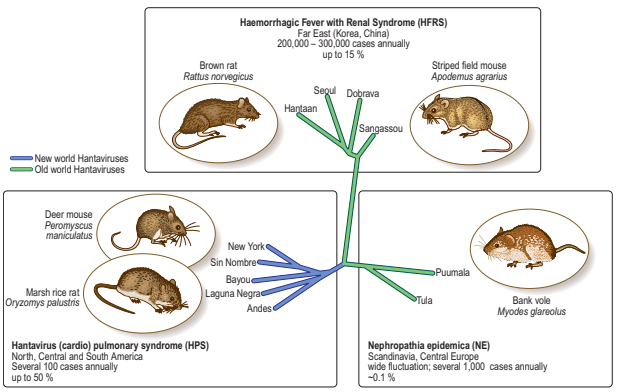

Fig. 3 **Important Old and New hantaviruses: their hosts and associated illnesses.**

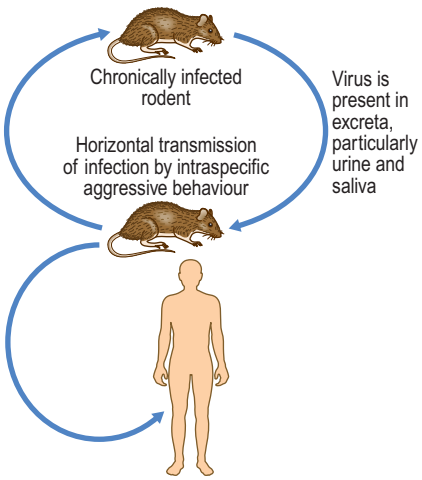

Fig. 4 **Hantavirus transmission. Infection occurs most commonly through inhalation of dust containing dried excreta from hantavirus-infected rodents.**

Orthomyxoviruses

The family *Orthomyxoviridae* contains three genera (or types): *Influenzavirus A, B,* and *C.* They are distinguished by their antigenically distinct nucleoprotein (NP) and matrix (M) proteins.

Influenzaviruses have a negative-sense single-stranded RNA genome which is segmented (influenza A & B: 8 segments; influenza C: 7 segments). The viral envelope contains two important surface glycoprotein antigens: haemagglutinin (HA or H) and neuraminidase (NA or N) (Fig. 1). The HA is responsible for cell attachment but it must first undergo post-translational cleavage by a trypsin-like protease into two peptides, HA1 and HA2, before the virus becomes infectious. The NA is enzymatically active and plays a role in virus maturation and release.

Influenzavirus nomenclature: type/location of isolation/consecutive number/year of isolation e.g. A/Sydney/5/97 [H3N2]

Influenzaviruses cause human disease in two different patterns:

1. Regularly occurring epidemics. In temperate and colder areas these epidemics occur during the cold season, in tropical areas anytime. They vary in magnitude.

   Seasonal (epidemic) influenza can be caused by influenza A or B viruses (influenza C virus is associated with common cold-like illness especially in children). As they circulate in the human population, these viruses undergo continuous, subtle antigenic changes in their surface antigens. This is termed 'antigenic drift' and is probably driven through pre-existing population immunity.

   Although most people will have some degree of pre-existing immunity stemming from previous infections, influenza is an important cause of morbidity and mortality in the high-risk groups. Antiviral drugs (adamantanes: amantadin, rimantadin; neuraminidase inhibitors: zanamivir, oseltamivir) are available but the most important prophylactic measure is vaccination. Inactivated (split virus or subunit) or cold-adapted live attenuated (FluMist) vaccines are available. They contain one influenza A H1N1, one influenza A H3N2 and one influenza B strain. Vaccination must be repeated annually, using vaccine that incorporates the strains most likely to circulate during the forthcoming season. A global surveillance network isolates strains from around the world to monitor these changes (Table 1).

   Clinically, influenza has been described as an 'unchanging disease due to a changing virus'. It is transmitted by respiratory droplets. After a short incubation period (c. 48 hours) onset is abrupt with fever, headache, photophobia, shivering, dry cough, malaise, myalgia, dry throat. Uncomplicated influenza lasts for about 1 week, but cough and weakness may persist for another few weeks.

   Complications are not uncommon and are more likely in infants, the elderly, and debilitated and chronically ill people (chronic airway disease; cardiac, hepatic or renal disease; diabetes; immunodeficiency). They manifest as secondary bacterial infections such as pneumonia, otitis media, sinusitis, rarely as primary viral pneumonia (often haemorrhagic), or myocarditis or encephalitis. Children with influenza who are given acetylsalicylic acid (Aspirin) can develop Reye's syndrome: encephalopathy and fatty degeneration of the liver, which carries a 40–50% mortality.
2. Occasional pandemics (global epidemics). These occur at irregular and unpredictable intervals: in 1918 ('Spanish 'flu'), 1957 ('Asian 'flu'), 1968 ('Hongkong 'flu') and 2009 ('swine flu').

   Pandemic influenza is caused only by a newly emerged 'human' influenza A virus subtype. The underlying mechanism is a major antigenic change, termed 'antigenic shift'. This stems from a process called 'reassortment'. If different influenzavirus strains infect the same individual, their replication may lead to the

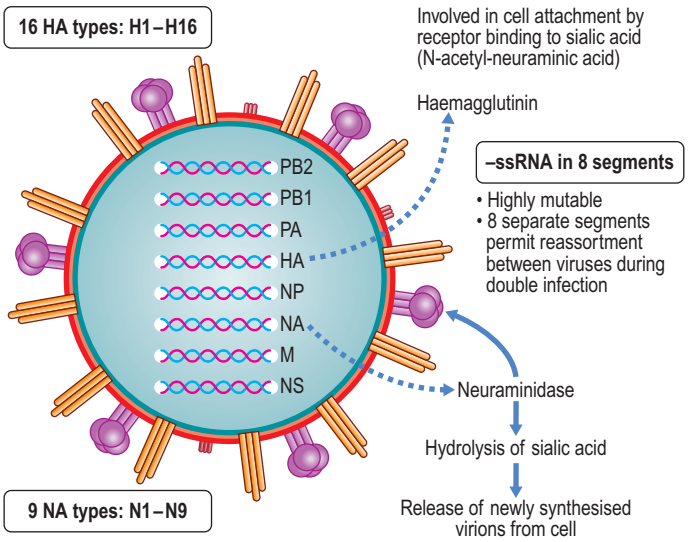

Fig. 1 **Structure of Influenzavirus A.**

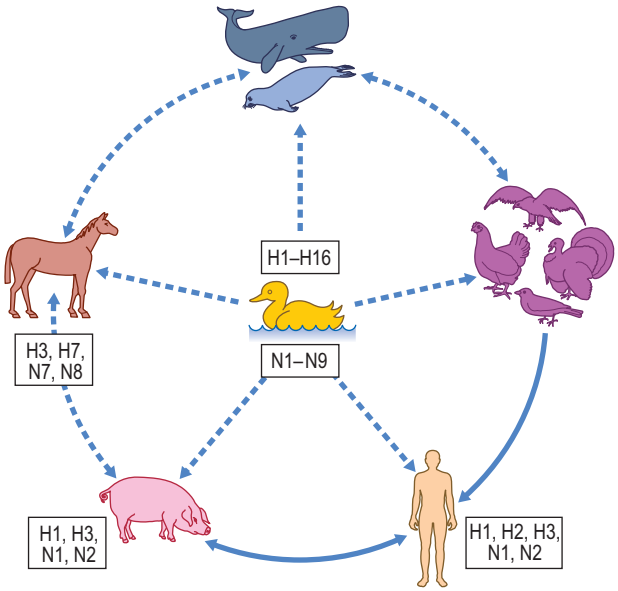

Fig. 2 **Influenza A viruses are essentially animal viruses.**

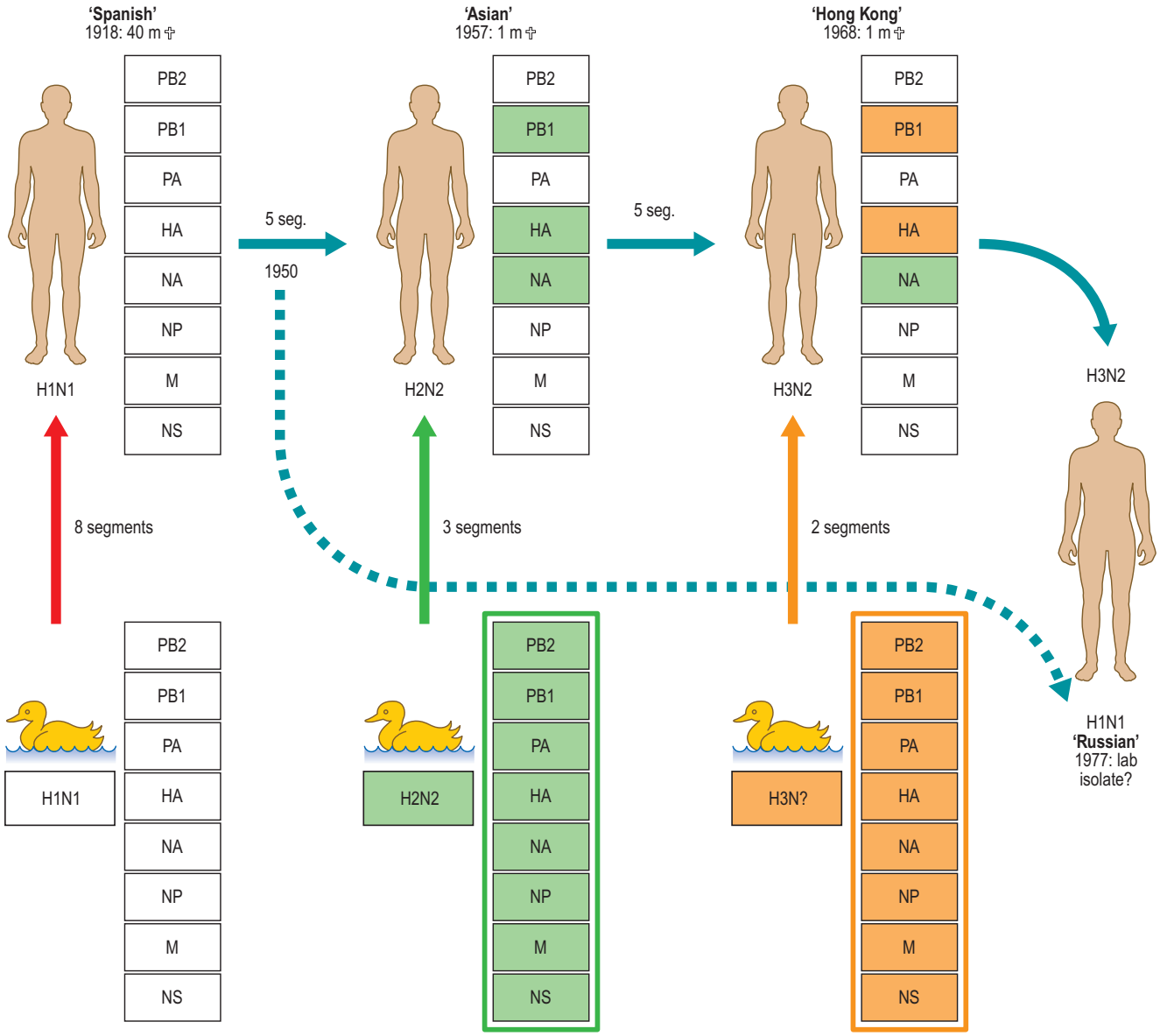

Fig. 3 **Influenza A viruses causing pandemics during the 20th century and how they emerged.**

emergence of progeny viruses whose genome segments come from different 'parent' viruses.

This may result in a virus strain with a haemagglutinin gene encoding a different subtype from those previously circulating in the human population. Thus there will be no population immunity against the novel virus strain, and if it is efficiently transmissible from human to human and has a high virulence, a pandemic will ensue. (Fig. 3).

Aquatic birds harbour a multitude of different influenzavirus strains (Fig. 2). They are, therefore, regarded as the original source for all influenzaviruses infection of pigs, horses, human beings and various other animal species. Reassortment between an avian and a mammalian virus strain can take place, for example in pigs, who then transmit this infection to humans; or it may take place in a human who is simultaneously infected with both a human and an animal virus strain.

Table 1 **WHO recommendations for seasonal influenza vaccines**

For the 2009/10 season (northern hemisphere):
■ A/Brisbane/59/2007 (H1N1)-like virus
■ A/Brisbane/10/2007 (H3N2)-like virus
■ B/Brisbane/60/2008-like virus
For the 2010 season (southern hemisphere):
■ A/California/7/2009 (H1N1)-like virus
■ A/Perth/16/2009 (H3N2)-like virus
■ B/Brisbane/60/2008-like virus
For the 2010/11 season (northern hemisphere):
■ A/California/7/2009 (H1N1)-like virus
■ A/Perth/16/2009 (H3N2)-like virus
■ B/Brisbane/60/2008-like virus

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Paramyxoviruses

Table 1 **Classification**

Order: Mononegavirales		
Family – Paramyxoviridae		
Subfamily	**Genus**	**Species**
Paramyxovirinae	Respirovirus	Human parainfluenzavirus 1 + 3
	Morbillivirus	Measles virus
		Canine distemper virus
		Rinderpest virus
	Rubulavirus	Mumps virus
		Human parainfluenzavirus 2 + 4
	Henipavirus	Hendra virus
		Nipah virus
Pneumovirinae	Pneumovirus	Human respiratory syncytial virus
	Metapneumovirus	Human metapneumovirus

Paramyxoviruses causing common respiratory infections: respiratory syncytial virus, parainfluenzaviruses 1–4, human metapneumovirus

Epidemiology

Respiratory syncytial virus (RSV), parainfluenzaviruses 1–4, and human metapneumovirus (hMPV) are common worldwide. Spread is via the respiratory tract, with contact with infectious secretions or aerosol and droplets. Respiratory syncytial virus is one of the most important causes of serious respiratory infections in the first year of life, and by 3 years of age most children have been infected. It is usually seen in winter. Outbreaks in day care centres and hospital wards occur easily, with a high attack rate. RSV also causes significant disease in the elderly and immunocompromised. Reinfection is common. Parainfluenzavirus 3 causes more severe lower respiratory tract disease in young children than types 1, 2, and 4, and is found all year round, with a peak in spring, whereas types 1 and 2 are seen mainly in autumn, and are mainly associated with laryngotracheobronchitis or croup. In 2001, human metapneumovirus was discovered. It causes seasonal epidemics starting in early spring, and is believed to contribute significantly to the respiratory illness burden of young children and the elderly, as a significant number of cases which test negative for other organisms are found to have hMPV. Respiratory disease that appears to have an infectious cause but where no causitive organism is found remains a diagnostic problem, and may be caused by as yet unidentified pathogens.

Pathogenesis

The virus enters via the respiratory tract, usually the eyes or nose, and replicates in the upper respiratory tract epithelium, from where it may spread from cell to cell to the lower respiratory tract. Airway inflammation and oedema occurs, with sloughing of epithelial cells, which may result in a ball-valve effect, causing air trapping, with hyperinflation.

Clinical picture

These viruses cause disease of the upper and lower respiratory tract, and the illness can range from mild to severe. For details, see the sections on respiratory tract illness.

Table 2 **Structure and replication**

Structure

Negative sense single stranded RNA
Genome 15000–16000 nucleotides long
Pleomorphic appearance, helical symmetry with envelope
150–400 nm in diameter
Surface proteins of importance: haemagglutanin-neuraminidase, fusion, glycoproteins

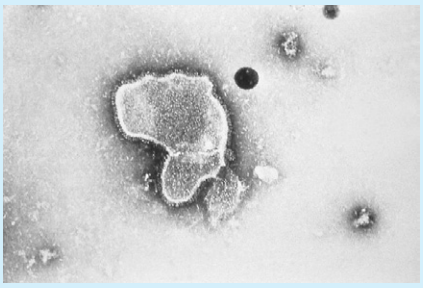

Fig. 1 **Respiratory syncytial virus.** (Photo courtesy of CDC/EL Palmer.)

Replication

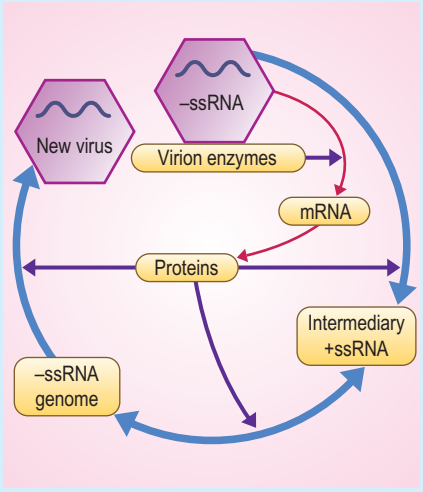

Fig. 2 **Replication of paramyxoviruses.**

Virological diagnosis

Serology: of little diagnostic significance; used for epidemiological studies and research.

Isolation: viruses are usually cultured from respiratory specimens on monkey kidney cells, and can also be detected by immunofluorescence on epithelial cells if the sample is of good quality. Rapid antigen testing for RSV is commonly used.

Molecular: the use of polymerase chain reaction (PCR), often as a panel of several viruses, is increasingly used for the diagnosis of all viral respiratory infections, but is not always available in all laboratories.

Specific treatment and prevention

Vaccines for RSV are still under investigation. Monoclonal antibodies, in the form of palivizumab, or RSV immunoglobulin, can be used to prevent RSV infection, and severe cases can be treated with ribavirin. Most cases of RSV infection, parainfluenzavirus and metapneumovirus infections are treated symptomatically and supportively. The diagnosis, however, is important as it can influence the

overall diagnosis and treatment. If clinicians are aware of the presence of a virus, they may choose not to use antibiotics, depending on further clinical evidence.

Measles

Epidemiology

The virus is spread mainly by aerosol droplets and respiratory secretions, which can remain infectious for several hours in the environment. Outbreaks are more frequent in winter, when it is less humid, and in tropical countries the timing of epidemics is less clear, but they tend to occur in hot, dry seasons.

Prior to the use of the vaccine, the age group most affected comprised children between the ages of 5 and 9 years, but today there are two main groups affected – those under 5 who have not been vaccinated and those between 5 and 19 where the vaccine has failed, due to inadequate vaccination, the presence of maternal antibodies at the time of vaccination or a drop in antibody over time. Today measles is rare, due to worldwide efforts. There are plans to eradicate measles eventually – there is an effective vaccine conferring life-long immunity, and there is no animal reservoir.

Pathogenesis

The virus enters the body through the upper respiratory tract or the conjunctiva, and initial replication occurs at the site of entry. From there the virus drains to the lymph nodes, and spreads to the rest of the reticuloendothelial system and respiratory tract. The incubation period is about 10 days.

Clinical picture

Prodromal symptoms include fever, malaise, conjunctivitis and upper respiratory tract symptoms, and sometimes a mild rash (urticarial or macular in nature) which disappears before the typical rash appears. At this point the patient is highly infectious. Koplik's spots may be seen, which fade about 2–4 days after the prodromal symptoms start and as the rash develops. As the rash develops, the symptoms worsen.

The rash starts behind the ears and on the forehead, and spreads to the trunk, and then to the rest of the body over about 3 days, after which it fades leaving a brown discoloration and fine desquamation. As the rash reaches its peak, the fever breaks, the symptoms subside, antibody titres rise and virus levels drop. Fig. 3 shows the rash of measles.

In patients who have received measles antibodies, either maternal or parenteral, or after live vaccine failure with only partial immunity, the symptoms may be present but reduced.

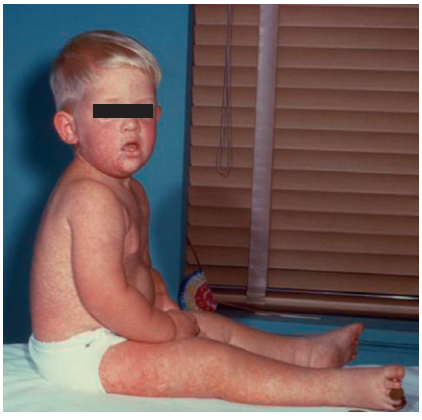

Fig. 3 **Measles.** (Photo courtesy of CDC.)

The measles virus inhibits cellular immunity, which may have clinical implications, such as in TB patients where TB may become significantly worse. This immune suppression may take as long as several months to recover. Intrauterine deaths have occurred due to measles, although it does not result in congenital abnormalities in those who are born after intrauterine infection.

Atypical measles

After incomplete vaccination, usually with inactivated vaccine or inactivated vaccine followed by live vaccine, a more severe form may be seen. (Inactivated vaccine was used in the past, and is no longer available due to this effect.) There is an incubation period of 7–14 days, followed by a headache, myalgia, abdominal pain and a high fever. The rash is not typical – it starts peripherally and spreads to the rest of the body. Initially it is a maculopapular and erythematous rash, but eventually becomes purpuric, vesicular or urticarial. It is present on the palms and on the soles. Most patients develop pneumonia.

Complications

Giant cell pneumonia

This is a disease seen in immune-compromised patients and is life threatening. Syncytia form in the lungs, alveolar cell proliferation occurs and squamous metaplasia of the bronchiolar epithelium takes place.

Measles inclusion body encephalitis

This is also a disease of the immune-compromised patient, common among children with leukaemia receiving radiation therapy, and also seen in HIV-infected persons. The incubation period can be a few weeks to 6 months and the symptoms start with convulsions, usually myoclonic jerks, that are mostly limited to one side. There may be hemiplegia or decreased consciousness. It leads to death within a few months.

Acute measles post-infectious encephalitis

This occurs in about 1 in 1000–5000 cases of measles, and among otherwise healthy persons. Fifteen per cent of cases are fatal and 20–40% of survivors are left with permanent neurological damage. It usually begins when the patient still has a rash, within 8 days of onset, but has been known to occur in the prodromal stage. Fever develops, with headache, seizures, ataxia and coma. The condition appears to be the result of an autoimmune reaction against the basic myelin protein.

Subacute sclerosing panencephalitis

This is a rare, progressive and fatal neurological condition that affects about 1 in 1 million patients who have acute measles. It affects boys slightly more than girls, and usually begins 6–8 years after the initial infection, but can sometimes take 20–30 years to develop. There is a higher risk among those who have measles before the age of 2 – half of all subacute sclerosing panencephalitis (SSPE) patients fall into this group. SSPE starts with a decrease in intellectual functioning or psychological problems, but at this point SSPE may not be suspected until the picture becomes clearer. Eventually visual and speech disturbances

develop, as well as myoclonic jerks and convulsions. EEG changes are characteristic – there are periodic slow wave complexes of high amplitude, which occur with myoclonic jerks, about 3–20 seconds apart. There is a high IgG level in the CSF. The reason for the development of SSPE is not yet certain, but it appears to have both virological factors, such as a deficient or mutated virus, and immunological factors to do with antibodies found in the CSF that appear to inhibit certain viral functions and not others.

Osteitis deformans and otosclerosis

It is unclear what role the measles virus plays in these conditions, but in patients with Paget's disease and otosclerosis, similar intranuclear and intracytoplasmic inclusion bodies, which react with anti-paramyxoviral antibodies, have been seen in osteoblasts, osteoclasts, fibroblasts and lymphomonocytes.

Virological diagnosis

Serology: the diagnosis of measles is primarily clinical. Serological methods, such as haemagglutination inhibition, testing for IgG titre rise or detecting IgM, may be of value.

Isolation: direct microscopy on respiratory cells can reveal giant cell formation and immunofluorescence microscopy on respiratory secretions and urine sediment, after removal of already bound patient antibody can detect viral antigen on cell membranes. Primary monkey kidney cells can be used to culture virus from respiratory samples, conjuctival washes or urine.

Molecular: polymerase chain reaction (PCR) may be of value, but is used mainly for research purposes. In cases of SSPE, RNA may not be present in the CSF, as the virus remains intracellular and can therefore not be cultured.

Specific treatment

Treatment is mainly supportive, with adequate nourishment being a key concern in developing countries. Immunoglobulin given in the first 3 days after exposure may prevent the disease or diminish the severity, but is of no value after 6 days. Antibiotics are appropriate with pneumonia, as it is difficult to distinguish between pneumonia due to measles and secondary bacterial infection. Anticonvulsants and fluid/electrolyte replacement are important for the management of measles encephalitis.

Prevention

The inactivated vaccine led to an increased incidence of atypical measles, due to immunity being incomplete. The live vaccines in use today (such as the Edmonston-Zagreb strain, and the Schwartz strain) are more attenuated and safer than the initial Edmonston strain. The incidence of encephalitis is 1 in 1 million recipients, whereas with the natural measles infection it is 1 in 1000–5000 cases.

Mumps

Epidemiology

Prior to the use of the vaccine, the highest incidence of mumps was in children between the ages of 5 and 9 years. However, because the disease is less contagious than other childhood diseases, many people only get it later in life, when they are more likely to be symptomatic – 90% of those between the ages of 10 and 14 are symptomatic, while all those over the age of 60 are. Some complications are more common after puberty – notably orchitis, oophoritis, and meningoencephalitis, the latter being 2–3 times more common in males than in females.

Pathogenesis

The primary site for replication is the mucosal epithelium of the upper respiratory tract and the eye. From there the virus spreads to the local lymphoid tissues and then the primary viraemia occurs, where the virus spreads to other organs – usually the parotid, but also the pancreas, testis, ovary and central nervous system. A secondary viraemia occurs, with further spread. Virus is excreted in urine and breast milk, but the main source of spread is via droplets from the respiratory system. Interferon appears to play a significant role in the pathogenesis and stimulates IgG, IgM, and IgA, as well as a cell-mediated response. There doesn't seem to be a higher risk for children with an immune deficiency.

Clinical picture

The classic picture is of parotitis, which occurs in 95% of symptomatic infections. Subclinical infections account for about 30% of all natural infections. The incubation period is 16–18 days. There may be a prodromal stage, with malaise, headache, fever and myalgia. Swelling of the one parotid gland is usually (in 75% of cases) followed by the other parotid gland between 1 and 5 days later. Sometimes other glands are involved, and sublingual and laryngeal swelling caused by lymphatic obstruction may cause problems. The parotid swelling subsides after about 7–8 days. Fig. 4 shows a child with parotitis.

Mumps was the predominant cause of aseptic meningitis prior to the general use of the mumps vaccine. The onset varies from 1 week before the onset of parotitis to 3 weeks afterwards. Symptoms subside 3–10 days later, and recovery is usually complete. Some patients (1 in 6000 mumps cases) also show signs of encephalitis, such as convulsions, abnormal movement, abnormal sensory perception and focal neurological signs. Sensorineural hearing loss is a less common complication of mumps, about 1 in 15 000 cases of mumps having permanent hearing loss.

About one-quarter of mumps cases in males after puberty include orchitis, with 20–40% of these being bilateral. There is acute pain and tenderness, with testicular enlargement. Nausea and vomiting may also occur. Late complications include infertility secondary to testicular atrophy. Oophoritis is less common in post-pubertal females than orchitis is in males, and it is not associated with infertility.

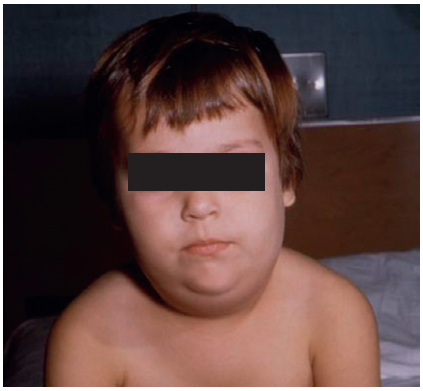

Fig. 4 **Mumps parotitis.** (Photo courtesy of CDC.)

STOP THINK

- What other childhood viral infections are preventable by vaccination?
- What other viral infections are easily spread in a hospital environment?

Other complications: involvement of other glands, such as the pancreas, prostate, lacrimal glands and other salivary glands; arthritis; myocarditis; transient renal dysfunction; nephritis; thrombocytopenia; spontaneous abortion (there is, however, no evidence of an increased risk of congenital abnormalities.)

Virological diagnosis

Serology: IgG and IgM; IgG levels correspond to levels of neutralising antibodies.

Isolation: mumps can be cultured from saliva and urine.

Molecular: PCR provides a more rapid diagnosis and is of use on CSF for a rapid diagnosis of meningitis.

Specific treatment and prevention

Treatment is symptomatic – control of fever and use of analgesics. Live attenuated vaccines, most of which are made from the Jeryl Lynn strain, are available alone and in combination with measles and rubella vaccines (MMR).

Henipavirus

Hendra and Nipah viruses are the two established species of the *Henipavirus* genus. Initially Hendra virus was discovered in Australia, followed by Nipah virus in Malaysia. Henipa-like viruses have recently been found in Africa.

Fig. 5 **Drawing by Gustav Mützel of a Large Flying Fox (*Pteropus vampyrus*), a type of fruit-eating megabat.**

Epidemiology

Henipaviruses appear to have flying foxes (large fruit-eating bats, see Fig. 5) and some other bat species as their natural hosts. They have a wide host range, and both Hendra and Nipah viruses can infect humans. Hendra virus was initially discovered in an outbreak in horses in Australia, with a very high mortality rate; Nipah virus was subsequently found in an outbreak in pigs in Malaysia and Singapore. The emergence of these viruses is likely due to increased agricultural encroachment of natural bat habitat such as fruit orchards. Human infection appears to be as a result of contact with sick farm animals, and not direct contact with the bat hosts. Human-to-human transmission is possible, especially where the infection results in respiratory illness.

Pathogenesis

The virus enters cells after binding to receptors on neurons, smooth muscle, and blood vessel endothelium, and this is followed by viraemia. Symptoms correlate well with the cell tropism, being mainly neurological and respiratory.

Clinical picture

Disease can be primarily respiratory or primarily neurological or a combination of the two. Nipah virus in humans usually causes a neurological infection, with 25% having accompanying respiratory symptoms. Presenting symptoms include fever, headache, depressed consciousness, and mild respiratory symptoms, and progression leads to worsening of respiratory and encephalitis symptoms, with pulmonary oedema/haemorrhage, difficulty swallowing or breathing, blurred vision, convulsions, coma, and often death. Survivors of Nipah infection may develop a delayed encephalitis.

Infection appears to be asymptomatic in bats, but mortality is high in both humans and domestic animals in the 40–75% range.

Diagnosis

Serology and PCR for Henipaviruses are not routinely available in most laboratories, and are usually done in specialised centres.

Prevention

Currently no specific treatment exists, and management is supportive. Ribavirin has shown limited success. Hendra virus vaccines for horses are in development.

Key points

- Measles is rare due to successful worldwide vaccination efforts.
- Measles virus and its vaccine are temporarily immunosuppressive.
- After puberty, complications of mumps can be more serious.
- Respiratory syncytial virus (RSV) is a significant cause of morbidity and mortality in infants.

Rhabdoviridae

Classification: family Rhabdoviridae, genus lyssavirus

Table 1 **Classification**

Genotype	Source	Distribution
Rabies virus	Dog, fox, raccoon, skunk, bat	Widespread
Lagos bat virus	Bats, cats	Africa
Mokola	Shrews, cats	South Africa, Nigeria, Cameroon
Duvenhage	Insectivorous bats	South Africa
European bat lyssavirus 1a and b	Bats	Europe
European bat lyssavirus 2a and b	Bats	Netherlands, UK, Switzerland
Australian bat lyssavirus	Flying foxes, insectivorous bats	Australia

Structure

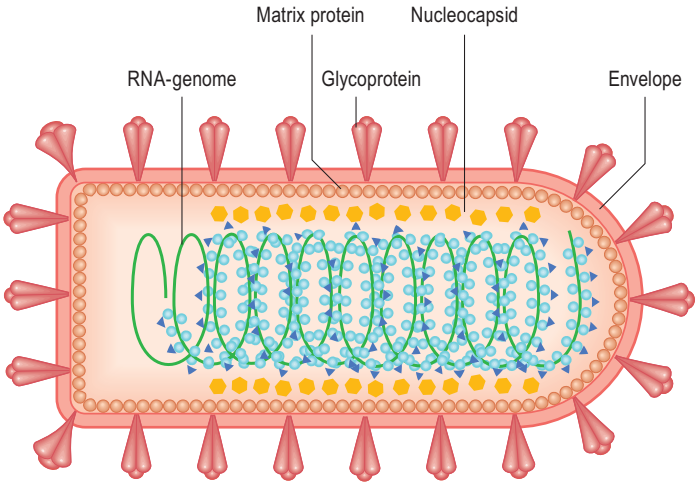

Fig. 1 **Structure of the rabies virus.**

Fig. 2 **Electron microscope photo of rhabdovirus.** (Photo courtesy of CDC/Fred Murphy.)

Epidemiology

Rabies is enzootic in many countries. Most cases, except in the USA, are due to rabid dog bites. Rabies can also be transmitted by foxes, raccoons, bats (USA), skunks, coyotes and wolves.

Transmission to humans usually occurs through the bite/lick of an infected animal. Iatrogenic transmission has been described in association with cornea and other solid organ transplants. Humans have contracted rabies by inhalation of vaccine preparations and possibly bat secretions in caves.

Pathogenesis

After entering through skin or mucosal membranes, the virus may replicate locally in the affected muscle before entering neurons or may enter the nervous system directly by binding to specific and non-specific receptors on the nerve cell e.g. the nicotinergic acetylcholine receptor at the neuromuscular junction.

The virus is transported to the central nervous system by way of retrograde axonal transport. Intraneuronal replication takes place in the dorsal ganglia and anterior horn cells. Rapid dissemination occurs within the CNS and the virus spreads centrifugally to peripheral sites, including salivary glands, lacrimal glands, adrenals, heart, lung and skin. No viraemia occurs.

Clinical picture in animals

Rabid animals can be recognised by abnormal behaviour including: irritability, restless wandering for miles, unprovoked attacks on humans, hypersalivation, dysphagia, tremors, paralysis of the jaw, neck or extremities and a change in the quality of barking. Death does not occur in all infected animals.

Clinical picture in humans

The incubation period of rabies in humans is usually 20–90 days, but may be as long as 19 years! Shorter incubation times have been observed in children, with bites to the head, neck and facial areas (a shorter distance to the CNS), with multiple bite wounds, a big viral inoculum and in bites to areas with high nerve densities.

Rabies encephalitis may be preceded by prodromal symptoms like fever, mood changes, agitation, fear, restlessness, irritability, insomnia, nightmares and depression. Patients may also complain of malaise, loss of appetite, headache, tiredness and a sore throat. Fifty percent of patients complain of pruritus and paraesthesia at the wound site. Tremors and fasciculations may be present. Rabies encephalomyelitis classically occurs a week after the prodrome. Two forms may present clinically. Furious rabies (excited, mad) is the most common form and is characterised by series of inspiratory jerky spasms in the respiratory muscles precipitated by the sight, thought and sound of water. This is termed *hydrophobia* (fear of water) and is pathognomonic of the disease. The spasms may also be triggered by bouts of airflow on exposed skin surfaces or *aerophobia*. The patient experiences excited as well as 'lucid' intervals. Widespread autonomic instability occurs and patients have increased secretion of saliva, sweat and tears. They may also experience transient elevations in blood pressure, tachycardia, ECG abnormalities, pupil defects and Horner syndrome.

Neurological manifestations may include convulsions, opistotonus, hallucinations, upper motor neuron signs, meningism, abnormal movements and coma.

The paralytic form of disease may include progressive ascending paralysis, sphincter dysfunction and inevitable respiratory paralysis.

CT and MRI scan can be normal, especially if taken early in disease.

Virological diagnosis

Table 2 **Virological diagnosis**

Specimens	Laboratory techniques
Animal ■ Brain in 50% glycerol – saline *Human:* Ante mortem ■ Cerebrospinal fluid ■ Saliva ■ Corneal scraping ■ Serum ■ Nuchal skin biopsies *Human:* Post mortem ■ Brain – brainstem, cerebellum, hippocampus in 50% glycerol saline	1. Electron microscopy 2. Tissue impression smears – Direct Ag detection/immunofluorescence 3. Culture/isolation: ■ Animal (mouse) ■ Cell lines – mouse neuroblastoma ■ Cells, baby hamster kidney cells 4. Molecular – polymerase chain reaction (PCR) 5. Serology – CSF and serum, as from week 2. 6. Histopathology

Table 3 **Risk categories for the administration of post exposure prophylaxis**

Risk category	Type of exposure	Action to be taken
1	Touching or feeding the infected animal Licking of intact skin	No action to be taken if history reliable, if not treat as for risk category 2
2	Nibbling of naked skin Superficial scratch without bleeding Licking of broken skin	Wound treatment – thorough washing, scrubbing and flushing with water, soap and a 70% ethanol/povidine iodine solution for >15 minutes. Avoid suturing and compressive bandages Rabies vaccine – stop vaccine course if animal is confirmed to be rabies negative No rabies immunoglobulin should be administered
3	Bites/scratches penetrating the skin and bleeding Licking of mucosal membranes	Wound treatment Anti-tetanus treatment/antibiotics if indicated Rabies vaccine – stop vaccine course if animal is confirmed to be rabies negative Rabies immunoglobulin indicated

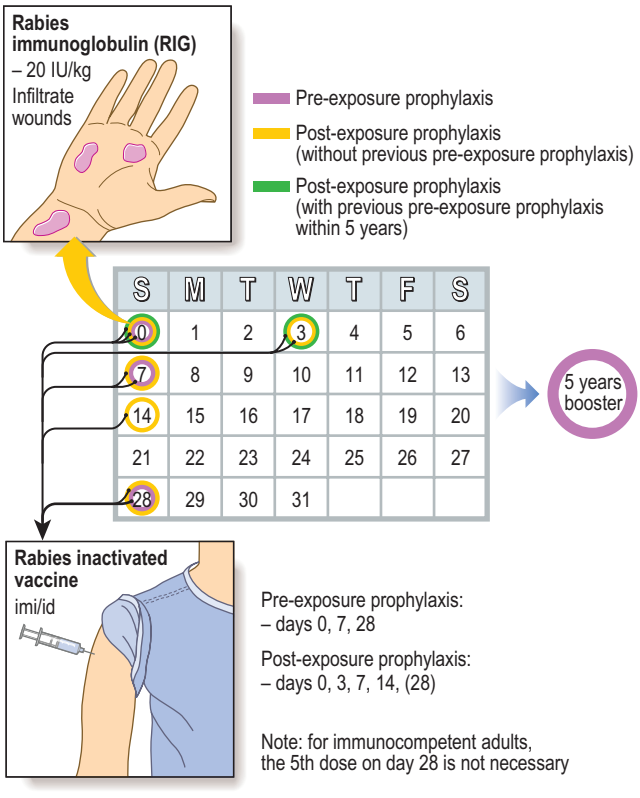

Fig. 3 **The timing of rabies prophylaxis.**

Treatment of rabies

Rabies has a 100% mortality rate when prophylaxis is not administered. Treatment is palliative and includes sedation and analgesia.

Prevention/prophylaxis

Various inactivated rabies vaccines are available and registered for both pre- and post-exposure prophylaxis. Cell-culture vaccines are registered for intramuscular as well as intradermal administration.

Pre-exposure prophylaxis is indicated in residents or travellers to endemic areas and in high-risk occupational exposure, e.g. laboratory workers involved in rabies research or vaccine development, veterinarians and animal handlers, or biologists. Pre-exposure prophylaxis simplifies the PEP regimes by eliminating the need for rabies immunoglobulin (RIG) and by decreasing the number of vaccine dosages needed.

See Table 3 and Fig. 3 for the indications and timing of rabies prophylaxis.

Doubling of the first dose of vaccine in rabies PEP is indicated in immunosuppressed and aged patients, patients with congestive heart failure, when a delay of more than 48 hours after exposure has occurred and when the patient has taken certain drugs, e.g. chloroquine, NSAIDs and steroids. Both rabies vaccine and immunoglobulin are considered safe in pregnancy and should not be withheld when indicated.

Verorab box. Photo courtesy of sanofi pasteur

Filoviruses

The Filoviridae form one of four families within the order Mononegavirales (the others are Bornaviridae, Rhabdoviridae – including rabies virus – and Paramyxoviridae – including measles virus and many others), characterised by their non-segmented, negative-sense RNA genomes. Under the electron microscope, filovirus particles appear pleomorphic: as thin, long filaments (Latin *filum* = thread), sometimes branched, or coiled giving them the characteristic appearance of a shepherd's crook.

Human filovirus infections are rare but spectacular events, often causing dramatic outbreaks with high fatality rates usually affecting health care facilities.

Onset of illness is abrupt. Starting with severe headache and malaise, patients develop severe haemorrhagic manifestations 5 to 7 days later, often bleeding from multiple sites. Case fatality rates vary from 25% to more than 80%, making filoviruses one of the most virulent human pathogens. In fact they are so virulent that outbreaks tend to 'run dry': Most infected individuals die too quickly to sustain ongoing transmission of the virus, while those who have escaped illness flee the scene in horror.

The first recognised filovirus outbreak occurred in Marburg and Frankfurt am Main, Germany, and in Belgrade, then Yugoslavia, in 1967: Monkeys freshly imported from Uganda fell ill and animal keepers, veterinarians and laboratory workers who had been in contact with diseased monkeys or their tissues and blood developed severe illness characterised by profuse bleeding.

Investigations revealed a hitherto unknown virus (Fig. 1) then named Marburg virus after the town from where it was first isolated.

In 1976, outbreaks of a similar haemorrhagic disease occurred in Yambuku, northern Zaire, and Maridi, southern Sudan (Fig. 2). The Zaire outbreak affected 318 patients with 88% mortality, the Sudan one 284 patients with 53% mortality. Both were caused by filoviruses; however these were distinct from Marburg virus and from one another and named Ebola-Zaïre and Ebola-Sudan viruses.

Since then, three further Ebola virus species have been identified:

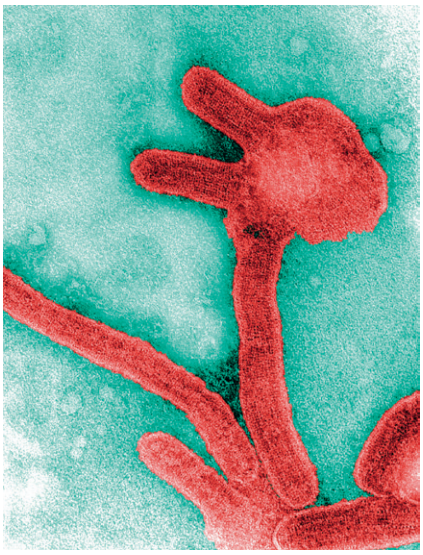

Fig. 1 **Colourised electron micrographs of Marburg virus particles taken in 1968 by F.A. Murphy.** (Photo courtesy of CDC/FA Murphy.)

Ebola-Côte d'Ivoire infected and sickened a Swiss scientist who had performed an autopsy on a dead chimpanzee in the Tai forest in Côte d'Ivoire in 1994; she survived and remains the only known human case. Ebola-Bundibugyo caused an outbreak with 93 cases in Uganda in 2007. Ebola-Reston is the only filovirus to originate from outside Africa, the Philippines. It is pathogenic for monkeys and pigs but not so far for human beings.

During several, often prolonged Ebola outbreaks in central African forests primates and other animals have died in great numbers. Butchering of affected animals may then lead to a spill-over into the human population, with subsequent human-to-human spread. The high virulence of Ebola disease in apes and other animals is of conservation concern but excludes them as plausible natural reservoir hosts. The likely scenario is that the apes contract infection through contact with the reservoir species, in the case of chimpanzees probably when they hunt other, smaller animals for food.

Many studies have attempted to identify the reservoir. Bats have been high on the list of suspects since the 1970s: Index cases of outbreaks had been in close proximity to bat roosts or visited caves used by bats. However it has only been over the past few years that surveys and experimental studies have demonstrated filovirus

Fig. 2 **Outbreak surveillance team travelling through the bush near Yambuku, Zaire (now Democratic Republic of Congo) during an Ebola virus outbreak in 1976.** (Photo courtesy of CDC/Joel Breman/Lyle Conrad.)

susceptibility and natural infection in fruit bats (flying foxes, family Pteropodidae) that are now believed to be the natural filovirus reservoir. Outbreaks of both Marburg and Ebola haemorrhagic fever seem to be increasing in frequency and magnitude; the reasons for this are unclear. Filoviruses are spread from person to person through contact with blood or other body fluids (faeces, vomitus, urine, saliva, respiratory secretions and semen) during the symptomatic phase of the illness but not during the incubation period. Guidelines have been published to improve infection control procedures in hospitals in developing countries, where sometimes blatant failures such as re-use of unsterilised needles have favoured nosocomial transmission. Management of cases is supportive; prevention of transmission is a main focus. Some experimental vaccines have shown promising early results in animal studies and may be available both for human beings and for rare and threatened wildlife species in years to come.

Further reading

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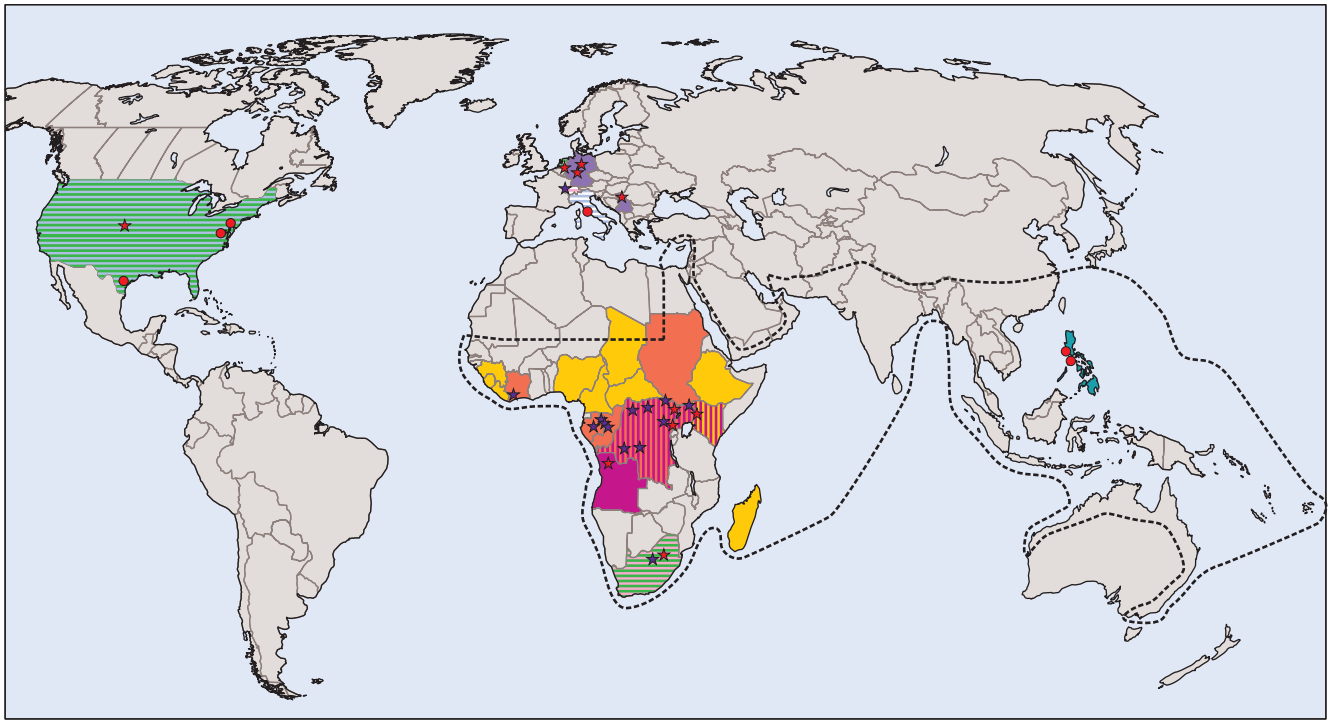


Location of reported Ebola Reston outbreaks in animals
Location of reported Ebola outbreaks or isolated cases
Location of reported Marburg outbreaks or isolated cases
Home range of Pteropodidae family of fruit bats
Countries with serological evidence of Ebolavirus
Countries reported Ebola imported case in human
Countries reported Ebola haemorrhagic fever outbreaks
Countries reported Ebola Reston outbreaks in imported monkeys from Philippines
Countries reported Ebola Reston outbreaks in monkeys and domestic pigs
Countries reported Marburg imported case in human
Countries reported Marburg outbreak following importation of infected monkeys from Uganda
Countries reported Marburg haemorrhagic fever outbreaks

Fig. 3 **Geographic distribution of outbreaks and cases of Ebola and Marburg haemorrhagic fevers and areas where fruit bats (family Pteropodidae) occur.** (Data from Global Alert and Response Dept., WHO.)

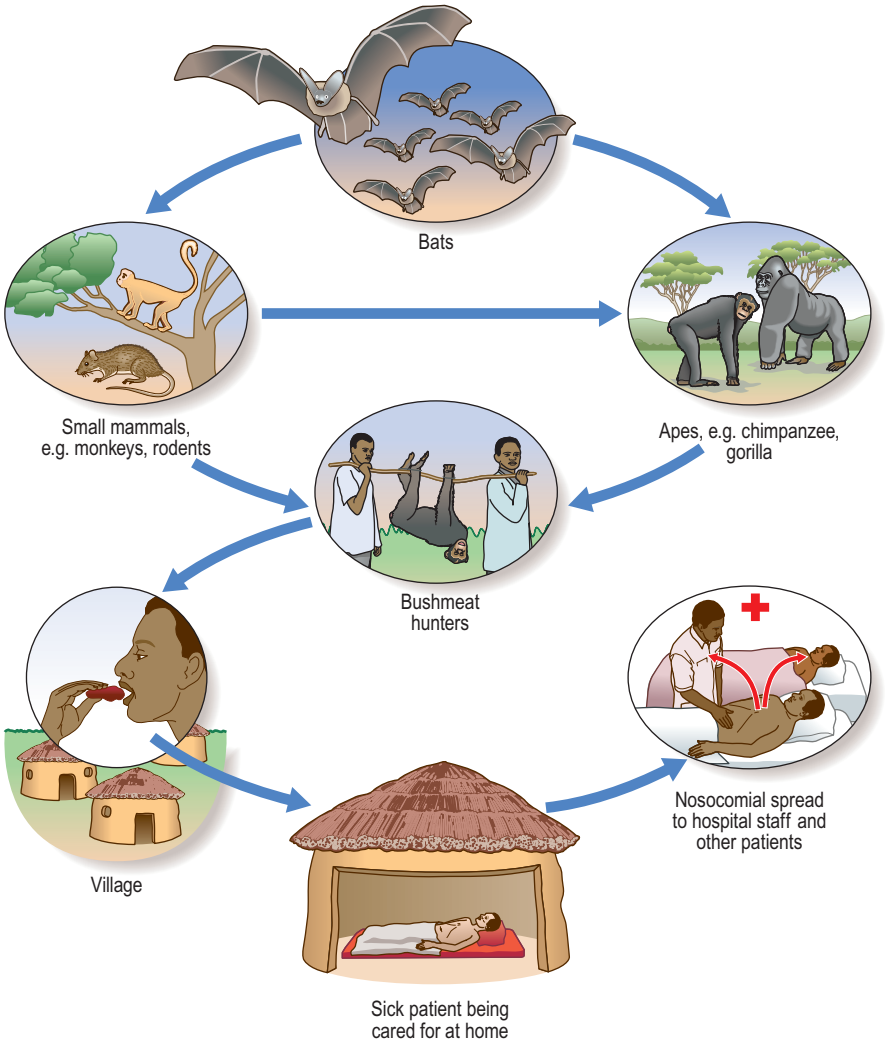


Fig. 4 **Speculated spread of filoviruses into the human population.**

Arenaviruses

Arenaviruses are divided into old world (Lassa fever, lymphocytic choriomeningitis (LCM)) and new world viruses (Junin, Machupo, Guarnarito and Sabia) viruses. They are known as arenaviruses because of the sandy appearance of the ribosomes which are acquired from the host cell (Box 1). Some arenaviruses are associated with person-to-person spread, e.g. Lassa and Machupo; whilst transmission of others is associated with contact with rodent excretions.

Box 1 **Features of arenaviruses**

Enveloped
Bisegmented single-stranded RNA
Cell cycle is restricted to the cytoplasm
Zoonotic diseases, generally associated with rodent transmission in humans.

The virus

Arenaviruses are pleomorphic in size ranging from 40 to more than 200 nm. The virion envelope is studded with evenly spaced glycoprotein projections (Fig. 1). These consist of complexes of glycoprotein. These viruses have a unique bisegmented ambisense linear RNA genome, comprising the L (large) segment which is about 7.5 kb and the S (small) segment which is 3.5 kb. This genome encodes for four proteins (Fig. 2). In common with other negative strand RNA viruses, the purified genome is not infectious.

Replication

Replication takes place in the cytoplasm. The arenavirus genome uses a unique ambisense coding strategy to direct the synthesis of two polypeptides in opposite orientation, separated by a non-coding intergenic region (IGR) with a stable hairpin structure. The viral RNA-dependent RNA polymerase (L) binds to a promoter on each segment, and transcribes a messenger RNA. Transcription is terminated by a strong hairpin sequence at the end of each gene.

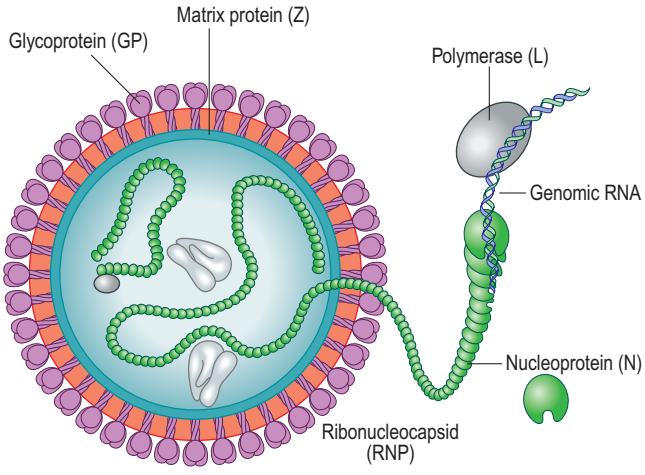


Fig. 1 **Structure of the arenavirus.**

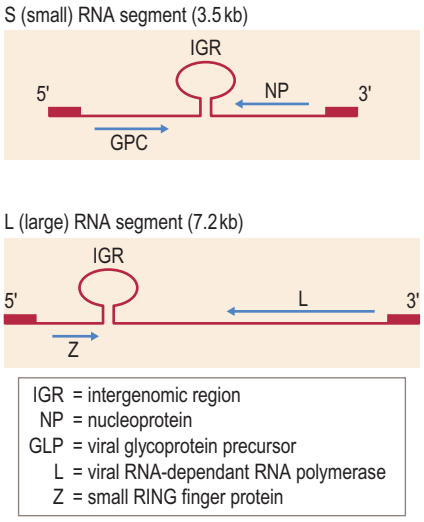


Fig. 2 **Schematic diagram of genome of arenaviruses.**

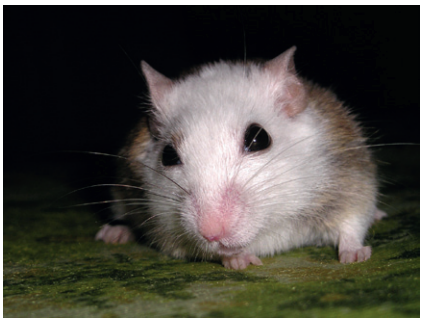


Fig. 3 **Multimammate mouse.** (Photo courtesy of Marek Herman.)

Replication is confined to the cytoplasm and budding takes place at the plasma membrane of the infected cells.

Lassa

Lassa is found in parts of West Africa. It is recognised in Guinea, Sierra Leone and Nigeria. The number of infections per year is estimated to be about between 100 000 and 300 000, with approximately 5000 deaths.

The host is a rodent known as the multimammate mouse of the genus *Mastomys* (Fig. 3). This rodent breeds frequently producing large numbers of offspring and is numerous in the savannahs and forests of West, Central and East Africa. They also readily colonise environments where humans live.

Transmission

Mastomys rodents shed virus in their urine and droppings. The virus may be spread through direct contact with these droppings, through touching contaminated objects, eating contaminated food or through contact with open sores. These rodents scavenge around food which has been poorly stored, facilitating transmission. Airborne transmission is reported has been described and occurs when viral particles are inhaled.

Lassa may also be spread through human-to-human contact. Contact with bodily fluids, blood tissue secretions

- What infection control procedures should be considered when managing a patient with suspected arenavirus infection?

or excretions. Transmission through contaminated medical equipment, e.g. reused needles is well known.

Clinical presentation

In endemic areas Lassa fever is mild or subclinical in 80% of cases, whilst in 20% of cases it causes a severe multisystem disease. The incubation time for Lassa is 1–3 weeks, after which the patient may report fever, chest pain, sore throat, cough, abdominal pain, vomiting, diarrhoea, conjunctivitis, facial swelling and mucosal bleeding. These symptoms are non-specific and may delay the diagnosis. The most common long-term complication is deafness. Death rates are particularly high for pregnant women, particularly in the third trimester, and 95% of fetuses die *in utero*.

Diagnosis

The virus can be detected in blood up to 14 days after onset of symptoms. The virus may be cultured in vero cells by dedicated laboratories and detected by immunofluorescence. More often Lassa virus antigen is detected by ELISA. This technique is robust and reliable. Reverse transcription polymerase chain reaction (RT-PCR) is another sensitive test used to detect the virus. Serology may be done by ELISA or immunofluorescence.

Treatment

Ribavirin has been used for treatment. It is most effective when given early in the course of the illness, together with supportive therapy.

Prevention

Prevention is achieved by avoiding contact with the mice, putting food away in rodent-proof containers, and avoiding consumption of these rodents. Nosocomial spread can be prevented through effective barrier nursing.

Lymphocytic choriomeningitis virus

LCMV was the first described arenavirus, isolated in 1933 during a study of an outbreak of St Louis encephalitis. It was the first recognised cause of aseptic meningitis in humans. LCMV has a worldwide distribution. Patients present with fever and about 10 days later present with meningeal signs. This may be associated with myalgia, prostration, nausea and vomiting. It is a rare cause of atypical pneumonia. Although this disease usually presents with meningitis, about 10% of cases have deeper neurological features. Encephalitis is reported in about 5–35% of hospitalised patients, but is rarely fatal in the immunocompetent (< 1%). The immunocompromised patient may have more severe disease. Other features include orchitis, parotitis, sudden onset deafness, thrombocytopenia or leucopenia. It is thought to be underdiagnosed in those with rodents as pets.

Diagnosis

The condition may be diagnosed by virus isolated from blood, antibody present in CSF which may be detected by ELISA or IFA.

Treatment

Treatment for LCM is supportive.

Junin and Machupo

Junin causes Argentine haemorrhagic fever (HF), whilst Machupo causes Bolivian HF. In areas which are endemic for these infections, subclinical infections are frequent. In those who present with clinical features of disease, the manifestations may be severe. The reservoir is the Vesper mouse of the genus *Calomys* found in South America. Man is only an incidental host. Those most commonly affected are male field workers who may come into contact with rodent excreta. The incubation time is 1–2 weeks. Primate studies suggest that viral replication occurs in the hilar lymph nodes and lungs shortly after infection. The subsequent viraemia causes dissemination to liver, spleen, heart and brain. The first signs are non-specific with malaise, fatigue, fever, headache and conjunctival injection. Patients may present during the second phase of the illness with a petechial rash, which may progress to florid bleeding, thrombocytopenia and neurologic manifestations. The case fatality rate is between 15% and 30%.

Diagnosis

Virus may be isolated from blood or tissue samples using tissue culture or suckling mice. RT-PCR techniques have also been developed.

Management

There are no known drugs that are effective against these viruses so treatment is supportive.

Table 1 **Old world and new world arenaviruses**

Old world	Distribution	New world	Distribution
LCM virus	Europe, Asia and Americas	Junin virus	Argentina
Lassa virus	West Africa	Machupo virus	Bolivia
Lujo virus, Mopeia virus	Southern Africa	Guanarito virus	Venezuela
Mobala virus	Central African Republic	Sabia virus	Brazil
Ippy virus	Central African Republic		

LCM, lymphocytic choriomeningitis virus.

Key points

- Arenaviruses are divided into old world (Lassa fever, LCM) and new world (Junin, Machupo, Guarnarito and Sabia viruses).
- Transmission may occur through person-to-person spread or through contact with rodent excrement.
- Treatment is supportive as no specific treatments have been developed for these infections.

Caliciviruses

Table 1 **Classification**

Family: Caliciviridae	
Genera within Caliciviridae	***Disease manifestations***
Norovirus	Associated with gastroenteritis outbreaks in humans. Five genogroups I to IV (I, II, IV gives human disease)
Sapovirus	Gastroenteritis in humans (childhood diarrhoea)
Lagovirus	Viraemia and liver necrosis and haemorrhagic disease in rabbits and hares
Vesivirus	Wide range of mucosal and systemic infections in animals

Structure and replication

Caliciviruses are non-enveloped, icosahedral viruses with a diameter of 27–40 nm (Fig. 1). The capsid consists of 90 dimers of the major capsid protein. The arrangement is such that 52 cup-like hollows on the surface are created which can be seen by electron microscopy (calici is derived from the Latin word for *calyx* which means cup). The genome is linear positive single-stranded RNA 7.4–8.3 kb in size. Non-structural genes for the helicase, protease and RNA-dependent polymerase proteins are at the 5′ end and structural proteins at the 3′ end of the genome. Little is known about viral receptor usage and cell tropism. Inside the infected cell two species of mRNA are formed by transcription through a complimentary (negative RNA) intermediate: a full genomic RNA which is translated to a polyprotein that is cleaved to form non-structural proteins and a subgenomic RNA of 2.2–2.4 kb that is translated into the major capsid protein. Capsid proteins self-assemble to form the capsid of the infectious virion.

Epidemiology

Two genera, norovirus and sapovirus infect humans and are referred to as human caliciviruses (HuCV).

Noroviruses are highly contagious since a low dose of only ten viruses can establish infection. Noroviruses are responsible for 68–80% of gastroenteritis outbreaks in industrialised countries. Transmission can be by contaminated food (such as oysters), water or surfaces contaminated with aerosolised vomitus, unwashed hands or person-to-person spread. Outbreaks can rapidly spread in enclosed communities, such as on cruise ships, and attack rates are about 50%.

Sapoviruses are associated with childhood diarrhoea, but can also cause diarrhoea outbreaks in day-care facilities, hospitals and schools. Occasional outbreaks in old-aged homes also occur.

Pathogenesis

People have different susceptibility patterns to different noroviruses and someone susceptible to the one may be highly resistant to the other. This is due to the fact that noroviruses infect enterocytes by binding to the H, Lewis and A histo-blood group antigens, which are highly expressed at the tips of the villi. Therefore, genetic differences in people affect their susceptibility to different noroviruses. Previous exposure to a particular norovirus can lead to an antibody (IgA and IgG) response that provides short-term protection to the same virus strain and probably partial protection to viruses of the same genotype, but not to other noroviruses.

Clinical picture

Incubation period of norovirus infections are usually 24–48 hours, but can be as short as 12 hours. It starts suddenly with nausea, vomiting, stomach cramps and watery diarrhoea, and in severe cases may lead to dehydration. Some people may have a low-grade fever. About 30% of people infected with noroviruses are asymptomatic. The duration of symptoms is usually 24–60 hours, but chronic diarrhoea can occur in immunosuppressed individuals. Patients under a lot of physical stress, such as in the military, may develop severe disease with systemic symptoms from norovirus infection.

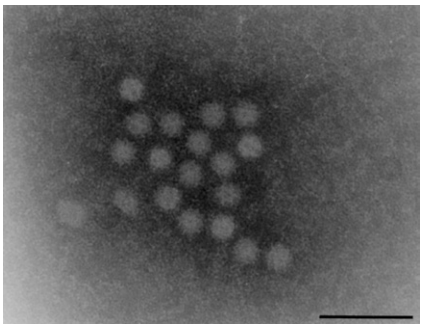


Fig. 1 **Caliciviruses in a stool specimen.** (Photo courtesy of Prof M Taylor, University of Pretoria.)

Virological diagnosis

In order to make a diagnosis one needs a diarrhoea stool sample. Diagnosis of HuCV previously depended on electron microscopy, which has recently largely been replaced by (the more sensitive) RT-PCR, which detects the presence of viral RNA and provides DNA that can be sequenced to determine the particular genotype of the virus. This has enabled the rapid tracing of outbreaks. Antigen assays, such as EIAs, to rapidly detect particular caliciviruses are now commercially available.

Specific treatment

No specific therapy is available. Supportive therapy of HuCV diarrhoea consists of fluid replacement.

Prevention

No vaccines or prophylactic chemotherapy are available.

Noroviruses can remain infective after freezing or temperatures up to 60°C. Noroviruses can also survive in chlorinated water. Important measures to prevent disease are the protection of public water systems by sewerage management and adequate sanitation; handwashing and good food preparation hygiene.

Astroviruses

Table 1 **Classification**

Family: Astroviridae	
Genus	***Species***
Mamastrovirus	Various species infecting many mammals such as feline and bovine astroviruses Human astrovirus (HAsV): 8 genotypes HAsV 1–8
Avastrovirus	Various species infecting birds

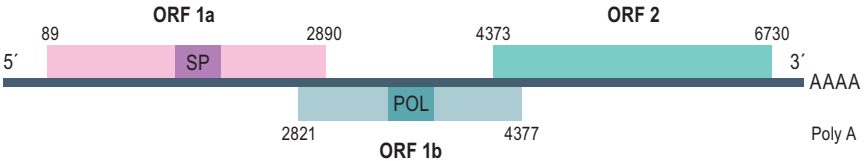


Fig. 1 **Genomic organisation of human astrovirus (HAstV), ORF-1a encodes a serine protease (SP), ORF-1b encodes an RNA polymerase (POL) and ORF-2 the structural capsid protein.**

Structure and replication

The name astrovirus is derived from the Greek 'astron' which means star and refers to the five- or six-pointed star appearance of the nucleocapsid as seen on electron microscopy of stool samples (Fig. 2). The virus genome is approximately 6.8 kb (excluding the polyA tail) single stranded positive RNA. It encodes three open reading frames (Fig. 1): ORF 1a (serine protease); ORF 1b (RNA-dependent RNA polymerase) and ORF 2 (structural or capsid protein). During transcription two species of RNA are formed – full genome, which is translated to form the non-structural proteins 1a and 1b. The 3′ end of the genome is transcribed to form a subgenomic RNA that is translated to the capsid protein. Little is known about receptor usage or cell tropism, except that enterocytes are likely infected by receptor-mediated endocytosis. Human astrovirus (HAsV) replication mainly takes place in the cytoplasm, but the nucleus and nucleoli play some undefined role.

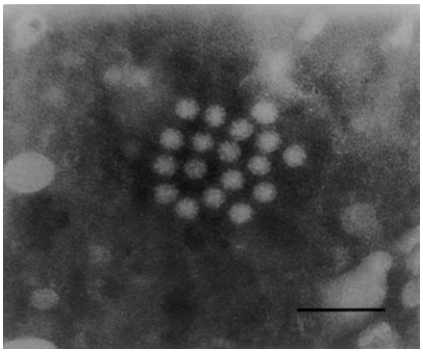


Fig. 2 **Astroviruses in a stool specimen.** (Photo courtesy of Prof M Taylor, University of Pretoria.)

Epidemiology

HAsV infections occur worldwide. They cause diarrhoea in young children and nosocomial diarrhoea, which can be especially severe in HIV-positive patients. It is the second most common virus found in children with diarrhoea, but it seldom results in hospitalisation. There is some evidence of seasonality in particular localities with predominance of outbreaks in spring and winter. HAsV infections can occur in many age groups but most infections occur in children between 2–4 years. The virus is faeco-orally transmitted and has been found in river water and shellfish. Person-to-person transmission is likely in hospitals and other care facilities. Large common-source outbreaks related to contaminated food or water also occur. HAsV are quite resistant to alcohol disinfection and can survive on surfaces, which also could play a role in hospital transmission.

Pathogenesis

The incubation period is 1 to 4 days. In an animal model, astrovirus was found to induce diarrhoea without causing cellular damage or inflammation. The exact mechanism of causing diarrhoea is unknown. The innate immune system, neutralising antibodies and CD4 T-lymphocytes seem to play a role in protection against infection.

Clinical picture

HAsVs cause a mild gastroenteritis usually lasting for 2–3 days. The virus can be shed for 2 weeks in normal healthy individuals and 3 weeks in immunosuppressed individuals. Astrovirus is the least severe of gastroenteritis agents. Co-infections with rotavirus and calicivirus make it difficult to establish the role of HAsVs in these cases.

Virological diagnosis

Stool specimens are required for diagnosis. Only about 10% of viruses will show characteristic morphology on electron microscopy. EIA to detect antigen is a more sensitive technique and there is a commercial assay available that can detect all eight human genotypes. Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive diagnostic technique.

Specific treatment

There is no specific treatment available. Most patients will recover spontaneously, whereas some patients will require fluid replacement.

Prevention

Decontamination of surfaces, hand washing and safe food-handling processes are important. Protection of water sources is also important, since astroviruses are relatively resistant to chlorination. No vaccine for HAsV exists.

Picornaviruses

Table 1 **Classification**

Family: Picornaviridae	
Genus	***Species***
Enterovirus	Human enterovirus A Human enterovirus 71 Human coxsackievirus A: 2–8,10,12,14,16 Human enterovirus B Human coxsackievirus B1–6 Human coxsackievirus A9 Human enterovirus 69 Human echovirus 1–7,9,11–21,24–27,29–33 Human enterovirus C Human coxsackie virus A1: 1,11,13,15,17–22,24 Poliovirus 1–3 Human enterovirus D Human enterovirus 68,70 Human enterovirus E
Rhinovirus	Human rhinovirus A + B + C
Cardiovirus	Encephalomyocarditis virus Theilovirus
Aphthovirus	Foot-and-mouth disease virus
Hepatovirus	Hepatitis A virus
Parechovirus	Human parechovirus Human parechovirus 1+2

Table 2 **Structure and replication**

Structure	**Replication**
Positive sense single stranded RNA Genome 7300 bases 27 nm diameter, icosahedral form Capsid proteins: VP1, VP2, VP3, VP4 Non-structural proteins: proteases, polymerase, nucleotide triphosphatase	

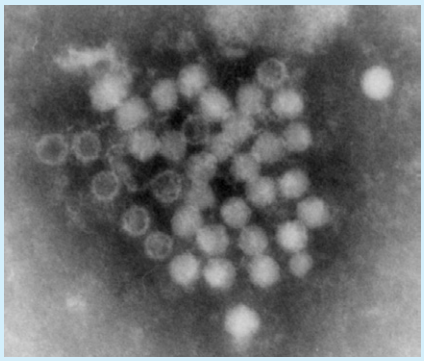


Fig. 1 **Enteroviruses. Note some viruses with empty capsids.** (Photo courtesy of Prof M Taylor, University of Pretoria.)

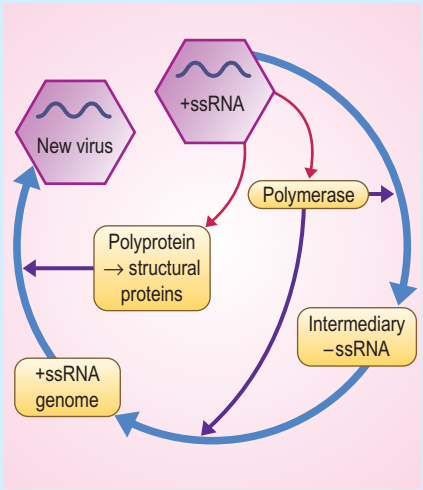


Fig. 2 **Enterovirus replication.**

Historically, enteroviruses have been classified into five groups – polioviruses, Coxsackie A viruses, Coxsackie B viruses, echoviruses and newly identified enteroviruses. A genome-based classification has replaced this.

Enteroviruses

The genome and replication

The enteroviruses use different receptors to bind to and infect cells, such as the Coxsackie-adenovirus receptor for Coxsackie B1–6. This explains the different tissue tropism that picornaviruses exhibit – e.g. liver for hepatitis A virus and nervous tissue for polioviruses. The genome is potentially infectious, as it can act as mRNA for the synthesis of viral proteins. See Fig. 2 for a representation of the replication cycle.

Epidemiology

Enteroviruses occur worldwide, although due to a campaign to eradicate polio, most countries are currently polio-free, with a few remaining with endemic polio, and some with imported cases resulting in outbreaks. Spread is largely faecal–oral due to shedding in stool, with some respiratory spread. In cases of conjunctivitis, direct inoculation is the means of spread.

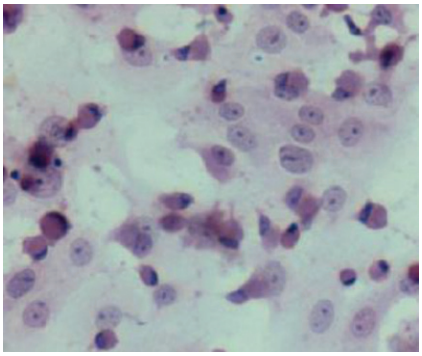


Fig. 3 **Cytopathic effect of Coxsackie virus B3 in primary monkey kidney cells. Note the large intracytoplasmic inclusion body pushing the nucleus to one side of the rounded cell.**

Pathogenesis

The virus enters via the oropharynx and replicates there and in the intestines. From there it spreads via the blood to a variety of other organs, depending on the individual virus. In the case of poliovirus, nervous tissue is infected, while Coxsackie viruses often infect the heart. Incubation periods differ widely between viruses and clinical presentations, ranging from 2 to 40 days.

Clinical picture

A wide variety of clinical pictures are caused by enteroviruses. The most well-known is poliomyelitis, which is caused by the polioviruses, with several other enteroviruses, most notably enterovirus 71, causing polio-like illness. Aseptic meningitis can be caused by all the enteroviruses, most commonly echoviruses, Coxsackie viruses B1-6, A7 and A9, and enterovirus 71. Encephalitis may also occur. Coxsackie B viruses are the most common cause of acute viral myocarditis, and persistence may result in chronic myocarditis or chronic dilated cardiomyopathy. Bornholm disease, or epidemic pleurodynia, infection of the intercostals or upper abdominal muscles, is also usually

- What makes a virus suitable for eradication?
- Why is it important to identify the cause of all cases of paralytic disease?

caused by Coxsackie B viruses, as is, along with several echoviruses, neonatal infection, which may range from a mild illness to fulminant multisystem disease. Outbreaks of such neonatal infection have been known to occur. Mild upper respiratory tract infections, herpangina and conjunctivitis with varying degrees of severity are also caused by enteroviruses. Hand, foot and mouth disease is associated with Coxsackie A viruses and enterovirus 71, while echovirus 9 can cause mild maculopapular rashes. Molecular mimicry – where a viral protein is similar to a human protein – can result in autoimmune disease, and enteroviral infections are thought to play such a role in the development of type I diabetes, although other factors, such as genetics, are also involved.

Virological diagnosis

Serology: this is labour intensive and involves the use of neutralisation assays to test for antibody levels. It is mainly used to test for immunity to poliovirus, and to diagnose recent infection with Coxsackie A viruses.

Isolation: Coxsackie A and B have been differentiated by different pathogenic effects in suckling mice. Coxsackie A viruses do not grow in cell culture, while monkey kidney or human cell lines can be used to culture the other enteroviruses. Fig. 3 shows the cytopathic effect of enteroviruses in primary monkey kidney cells. Different types can be differentiated by immunofluorescence testing of infected cells, or by neutralisation assays, where antibodies preventing infection by individual enteroviruses are added to cultures to see which antibodies inhibit viral replication.

Molecular: polymerase chain reaction (PCR) is now frequently used to diagnose enterovirus infection, and can differentiate between types. It is faster than isolation.

Specific treatment

Pleconaril is a drug that binds stably to the virion, preventing cellular attachment; however, it is not routinely available.

Prevention

For polioviruses 1–3, there are live attenuated (Sabin) and inactivated (Salk) vaccines available. Both are usually given in multiple doses, as inactivated vaccines often require booster doses and the live vaccine strains can interfere with each other's replication. Worldwide vaccination against polio has reduced morbidity and mortality tremendously, and eradication is expected in the near future. For other enteroviruses, no vaccines are available.

Rhinovirus

Epidemiology

There are over 100 serotypes of rhinovirus and they are found worldwide. They cause common respiratory infections in all age groups. Spread is via the respiratory route. They do not cause severe disease, but the worldwide economic impact is considerable.

Pathogenesis

The virus enters via the nose and replicates locally in the epithelium of the nose and nasopharynx. Occasional lower respiratory tract replication has been observed. The immune response triggers increased nasal secretions and irritation, which result in the symptoms. Viral clearance follows.

Clinical picture

Rhinoviruses are an important cause of rhinitis and otitis media, and may also cause bronchiolitis and pharyngitis.

Virological diagnosis

Serology: not used for diagnostics due to the large number of serotypes.
Isolation: labour intensive and of no clinical value. Most diagnostic methods are used mainly in research and epidemiological studies.
Molecular: the most practical form of diagnosis is by PCR, but this is usually unnecessary.

Specific treatment and prevention

Treatment is symptomatic. Hygiene plays an important role in avoidance of rhinovirus infection.

Hepatovirus

The genus hepatovirus contains one species, the hepatitis A virus. This is discussed in detail in the chapter on viral hepatitis.

Other picornaviruses

Of significance are the three parechoviruses, which can cause mild respiratory and gastrointestinal disease, and occasionally neurological disease – flaccid paralysis or encephalitis.

Key points

- Poliomyelitis can be successfully prevented by vaccination against poliovirus.
- Diagnosis of mild viral upper respiratory tract infections is usually not necessary.
- Hygiene plays an important role in the prevention of most picornavirus infections.

Human coronaviruses

Table 1 **Classification**

Order: Nidovirales	
Family: Coronaviridae	
Genus	**Species**
Coronavirus	Human coronavirus 229E
	Human coronavirus OC43
	Human coronavirus NL63
	Human coronavirus HKU1
	Severe acute respiratory syndrome coronavirus
	Human enteric coronavirus
Torovirus	Human torovirus

Table 2 **Structure and replication**

Structure

Positive sense single stranded RNA
Genome ~30 000 nucleotides long
Pleomorphic viruses
80 × 160 nm diameter, with 12–24 nm surface projections (spikes) that cause the corona (Latin: crown) appearance
Major proteins:
S – spike
E – envelope
M – membrane
N – nucleocapsid

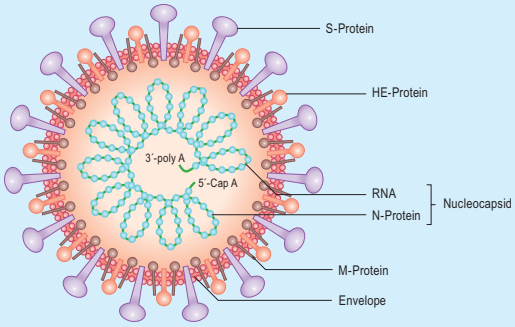


Fig. 1 **Coronavirus.**

Table 3 **Structure and replication**

Replication

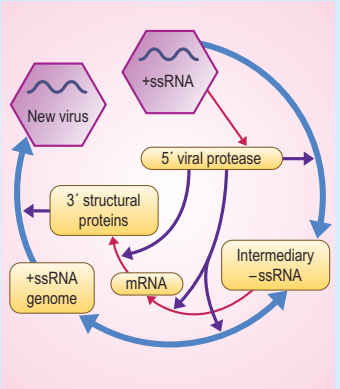


Fig. 2 **Replication cycle of coronaviruses.**

History

Coronavirus disease was first described in 1931, with the first coronavirus (HCoV-229E) isolated from humans in 1965. Until the outbreak of severe acute respiratory syndrome in late 2002, only two human coronaviruses (HCoV) were known – HCoV-229E and HCoV-OC43. Once the SARS coronavirus (SARS-CoV) had been identified, two further human coronaviruses were identified. Three groups of coronaviruses exist: group 1 (HCoV-229E and HCoV-NL63), group 2 (HCoVOC43 and HCoV-HKU1), group 3 (no human CoVs as yet). SARS-CoV is an outlier to all three groups, although some place it in group 2.

Group 1 and 2 coronaviruses

Epidemiology

All four of these human coronaviruses have been reported from around the world. Most studies have focused on children, where disease is more significant, although they infect adults as well. Infections are seen throughout the year, although more often in winter and spring, with larger outbreaks every 2–4 years.

Pathogenesis

Viruses enter the respiratory tract, where they replicate in the epithelial cells of the upper respiratory tract. Spread to the lower respiratory tract occurs.

Shedding of virus is from the respiratory tract during acute infection, and virus may continue to be shed for some time from the gastrointestinal tract after recovery.

Clinical picture

Coronaviruses are an important cause of the common cold – 2–10%, second after rhinoviruses. In adults, the illness is usually limited to common cold symptoms of rhinitis, sore throat and sometimes coughing. In asthmatic patients, and patients with chronic bronchitis and other chronic lung diseases, the underlying illness may be worsened.

In infants, the infection can be more severe, causing tracheolaryngobronchitis (croup), bronchitis and pneumonia.

Diagnosis

Due to the mild and passing nature of the illness, diagnosis is seldom required and is usually limited to diagnosis in the recovery period or of past infections in epidemiological studies. Few laboratories test for coronaviruses, and fewer offer such testing as a diagnostic service. In more severe cases of infection in infants, many other more treatable causes are usually excluded first. The future may hold easier access to rarer diagnostic tests such as these:

Serology: this is of little clinical use; it is used for epidemiological purposes.

Isolation: cell culture, e.g. vero cells.

Molecular: polymerase chain reaction (PCR) – usually used in specialised centres.

Specific treatment

There is no specific treatment for coronaviruses. Treatment is symptomatic and supportive.

Prevention

General hygiene and disinfection can prevent person-to-person spread. No vaccine is available.

Severe acute respiratory CoV

Epidemiology

Between November 2002 and July 2003, 8096 probable SARS cases were reported to the World Health Organization. The total number of deaths rose to 774, with a case mortality rate of 9.6%. Since then, three

STOP THINK

- What other infectious agents cause epidemics and pandemics?
- What clinically relevant new viruses have been discovered recently?
- In which other infections does the immune response play an important role in causing pathology?

laboratory-associated outbreaks occurred, with a total of 11 cases. Since then SARS has not been circulating.

SARS-CoV entered the human population from an animal source – several animals have been found to have very closely related viruses. The epidemic began in Guangdong province, China, from where it spread to Vietnam and Hong Kong, and then to other countries. Spread of the virus was via respiratory droplets and, therefore, contact with surfaces was important. Since it was excreted via the gastrointestinal tract, faecal–oral transfer was a possibility. Certain individuals, called super-spreaders, were more infectious than others, and are thought to have been responsible for most cases of transmission. The incubation period was 4–6 (range 1–14) days and patients were only infectious during the symptomatic period and most infectious during the second week of illness.

Pathogenesis

Determining the pathogenesis of SARS was difficult, based on autopsy findings confused by the effects of treatment, e.g. ventilation and medication. The virus replicates in the lower respiratory tract, followed by an innate and a specific immune response, and both viral factors and immune response (e.g. cytokine dysregulation) play a role in the pathogenesis. The first stage of the disease is associated with diffuse alveolar damage, macrophage and T-cell infiltration, and type 2 pneumocyte proliferation. The pulmonary infiltrate appears patchy on the chest X-ray. In the second stage, organisation occurs. The infection is not limited to the pulmonary system. The virus replicates in enterocytes, resulting in diarrhoea, and is shed in the stool, as well as urine, and possibly other body fluids.

Clinical picture

The first phase of the disease consists of fever > 38°C with rigors, myalgia, sore throat and gastrointestinal symptoms, with cough and often shortness of breath beginning after about 3–7 days of symptoms. Hypoxia may develop and 10–20% require ventilation. A relative lymphopenia and neutropenia may occur. Some patients showed a biphasic course, with apparent recovery followed by worsening of the clinical condition. Mortality was highest in the elderly and lowest in the younger population, and co-existing illness worsened morbidity and mortality.

Diagnosis

Initially, the aetiological agent had to be identified, and this was done rapidly, through a concerted effort of scientists from around the world, using a combination of: serological techniques, viral culture, electron microscopy, histopathology and molecular methods to narrow the list of potential agents down to the point where the specific coronavirus was known. This was a remarkable case study in the identification of an unknown organism.

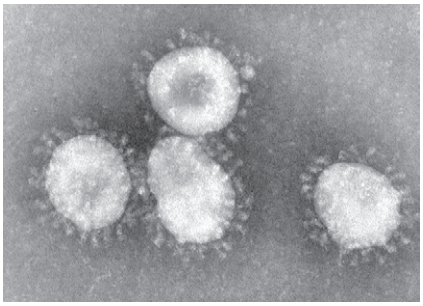


Fig. 3 **Coronaviruses as seen under an electron microscope.** (Photo courtesy of CDC/Fred Murphy.)

Important in the individual patient's diagnosis of SARS was the exclusion of other organisms that could cause similar pathology, as well as the establishment of an epidemiological link:

Serology: presence of antibodies indicated infection, and formed part of the diagnostic criteria (a four-fold rise in titre, or seroconversion).

Isolation: electron microscopy (Fig. 3).

Cell culture: vero cells.

(Diagnostic criteria required confirmation by polymerase chain reaction (PCR) to identify the virus as SARS-CoV.)

Molecular: reverse-transcription PCR (two or more primer sets on the same positive sample or two or more positive samples were required in the diagnostic criteria).

Specific treatment

Supportive treatment, such as ventilation, was the centrepiece of the management of a SARS patient. Specific treatments, such as steroids, remain controversial regarding their effect, whether adverse or beneficial, on SARS patients. Ribavirin was used, as it is a broad-spectrum guanosine analogue, and appeared to have some benefit, as did other agents that have an effect on the inflammatory response, such as chloroquine and interferon, and agents that interfere with coronavirus protease activity, such as the lopinavir/ritonavir combination used for HIV treatment. SARS-specific immunoglobulin was also tried. The degree to which any of these was successful as treatment is still debated.

Prevention

Isolation of infected patients and quarantine of exposed people, along with disinfection and infection control precautions, were the key to prevention of spread. A vaccine has been investigated, but may be merely an academic measure, relevant to future vaccine research, since the epidemic is over and SARS is no longer circulating.

Key points

- Severe acute respiratory syndrome (SARS) is no longer circulating in the human population, but laboratory stocks exist.
- Coronaviruses are common causes of colds in adults and important causes of croup and lower respiratory tract infection in infants.
- Facilities to detect and diagnose coronavirus infection are scarce.

Flaviviruses

Note: Over seventy members of the family *Flaviviridae* are responsible for human disease. Only some important ones are discussed here.

Table 1 **Classification**

Genus	Species
Flavivirus	Many arboviruses
Pestivirus	Infect non-human mammals
Hepacivirus	Hepatitis C virus, GBV-C

Table 2 **Important human arboviruses from flavivirus genus**

Tick-borne viruses	Tick-borne encephalitis Omsk haemorrhagic fever Powassan	Various geographical subtypes
Mosquito-borne viruses	Dengue virus group Japanese encephalitis (JE) group Yellow fever (YF) virus group	Dengue virus 1–4 JE, St Louis encephalitis virus (SLE), West Nile virus (WN) YF virus, Wesselsbron virus

Structure and replication

Flaviviruses (FVs) are 40–60 nm in diameter, round with a lipid envelope. The capsid consists of many copies of a single capsid protein. Two to three virus proteins are embedded in the membrane. FVs contain a single-stranded positive RNA genome. Fig. 1 shows the genome structure and Fig. 2 illustrates the replication of FVs.

Epidemiology

The main routes of transmission for human FVs are either by blood or blood products (hepatitis C virus or any of the arboviruses during the viraemic phase) or by arthropods–mosquitoes and ticks. For more detail on hepatitis C virus see the chapter on hepatitis viruses. The rest of this discussion will focus on arboviruses, which needing an arthropod vector to be transmitted from one animal to the other, are adapted to replicate in these two very different host systems. Some of these FVs can also be transovarially or sexually transmitted between insects – with the result that insects and animals can act as reservoir.

Some viruses are highly host-specific and others infect many different animal species.

The range of many of these infections expanded during the previous century. Dengue was initially found in South East Asia, but it is now found throughout the tropics. Japanese encephalitis (JE) was initially found in the temperate areas of East Asia has spread to South East Asia and South Asia by 1970. West Nile (WN) was restricted to Africa and Eurasia, but was introduced into the Americas in 1999 where it spread extensively.

Yellow fever virus (YF) shows two epidemiological cycles: an arboreal and an urban transmission pattern. In the arboreal non-human cycle primates are the reservoir and humans entering the jungle are accidental hosts when bitten by tree dwelling mosquitoes. In the urban transmission cycle, humans act as the reservoir and infection is transmitted by the anthropophilic *Aedes aegypti* mosquito. For FVs, excluding dengue virus infection and urban YF, human hosts are not important reservoirs, but are usually dead end hosts. However, rare cases of human-to-human transmission during organ transplantation and blood transfusion have recently been reported for WN virus. Also rare cases of in-utero transmission have been recorded for JE, WN and dengue, and breast-milk transmission of WN.

In the case of JE the virus is carried over large distances by herons and other birds. The infection is then transmitted by mosquitoes to pigs, acting as amplifying hosts. *Culex* and other mosquito species again transmit the infection from pigs to humans.

Birds (especially water birds) are the natural reservoir of WN and SLE. These viruses now share more or less the same geographical distribution in North America. *Culex* mosquitoes are the predominant vectors for both these viruses.

Environmental factors such as global warming, together with the breakdown of vector control in tropical countries, may result in the wider distribution of these arboviruses in the future.

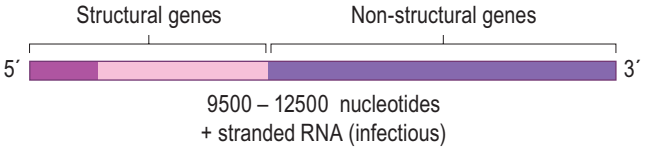


Fig. 1 **Genome structure of flaviviruses.**

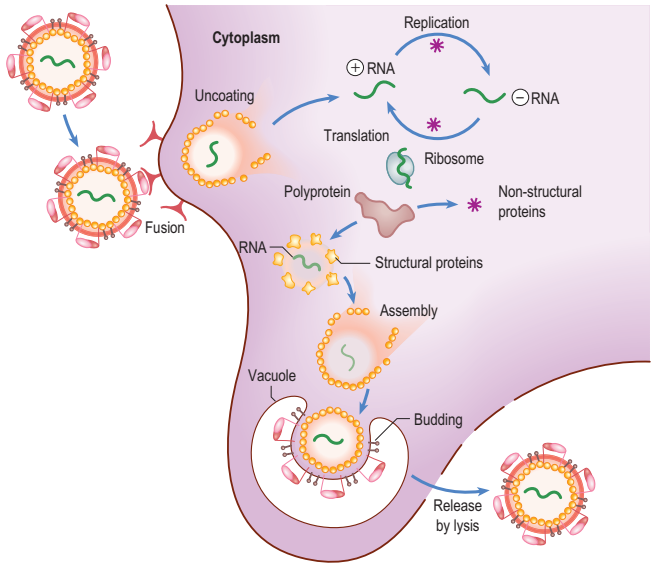


Fig. 2 **Replication of flaviviruses.**

Pathogenesis

Yellow fever virus

After inoculation YF replicates in the regional lymph nodes and then spreads to the reticuloendothelial organs and muscle tissue. Hepatocytes

are the most important target cells and damage to the liver is by direct cytopathic effect. The pathogenesis of renal involvement is unknown. In severe cases bleeding tendency results from a lack of clotting factor synthesis by the liver which can be complicated by disseminated intravascular coagulation and thrombocytopenia.

Dengue virus

The primary target cells of dengue are dendritic cells and monocytes. Antibodies to dengue virus NS1 protein cross react with endothelial cells and platelets and are implicated in increased vascular permeability and development of thrombocytopenia. Non-neutralising heterotypic antibodies are involved in disease enhancement if secondary dengue infections occur with a different type. These antibodies enhance infection of monocytes by binding to Fc-receptors resulting in an increased risk of dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS), which are the most severe complications. Genetic factors seem important since there is an association with certain HLA types and an absence of DHF or DSS in black people from Haiti and West Africa despite the fact that these are hyper-endemic areas. Viral virulence factors, related to the ability to escape the innate immune system and selectively down-regulate interferon α, may play a role. Bleeding-tendency in DHF probably results from thrombocytopenia (decreased production and increased consumption due to endothelial damage) and consumptive coagulopathy. Shock in DSS is due to leakage of fluid from the vascular compartment into the interstitium.

Flavivirus encephalitis and myelitis

Viruses can reach the CNS either by direct neuronal spread or by blood stream (haematogenous) spread. Haematogenous spread is the most important in cases of arboviral infection of the CNS. Neurogenic spread via the olfactory tract possibly plays a role in St Louis encephalitis virus (SLE) infection. Viruses first replicate at the site of inoculation (skin) from where they spread to the blood to form a primary viraemia. From there they spread to the reticuloendothelial system (RES) where further amplification occurs. Secondary viraemia follows, infecting the capillary endothelia of the cortical vessels. From here the CNS is invaded with infection of neurons. CNS pathology is characterised by perivascular lymphoid infiltration that can in prolonged cases be followed by astrocytosis and glyosis. A specific IgM response is associated with clearance from the CNS and clinical recovery. The cellular immune system is also thought to play an important role since organ transplant patients have been reported to suffer severe WN encephalitis.

Clinical picture

Yellow fever

YF has an incubation period of 3–6 days. It is a biphasic illness with the first part non-specific: headache, fever, malaise, nausea and muscle pain (especially back pain). Most patients recover after the first phase, but in severe YF it is followed by a brief recovery and then the return of fever, vomiting, abdominal pain, bleeding tendency, jaundice and renal failure. Biochemical investigations show elevated bilirubin and transaminase enzymes. When the second phase illness occurs the mortality is high.

Dengue virus infection

There are three clinical pictures: classical dengue fever, characterised by fever, frontal headache, nausea, vomiting and severe muscle-and bone pain; DHF: classical dengue with bleeding tendency and DSS which is characterised by hypotension due to vascular leakage.

Flavivirus encephalitis

Disease is more severe in the old aged (SLE, WN and JE) or very young (JE). There is a wide variation in the severity of disease among individuals. Tick-borne encephalitis (TBE) may show a biphasic pattern but in many cases of FV encephalitis signs of CNS involvement can be present with onset of illness. Early on in the disease course CT scan or MRI may be normal and later areas of focal necrosis may be seen. FV encephalitis often involves the basal ganglia and brainstem. Polio-like myelitis has been found with TBE and WN.

Virological diagnosis

Serological tests employed are IgM capture ELISA, haemagglutination inhibition (HI), immunofluorescence (IF) and compliment fixation (CF). IgM capture ELISA is the most sensitive serological test early in disease. For diagnosis of FV encephalitis serology on CSF is performed. Problems with serology are that IgM may be negative early in disease, diagnosis with HI, IF and CF is retrospective since a four-fold rise in titre is required for diagnosis. Endemic FVs may result in cross-reacting antibodies and should also be tested – the causative virus should show higher titres. Various mammalian and insect cell-lines are available for isolation of virus from specimens such as CSF. Reverse transcriptase polymerase chain reaction (RT-PCR) under correct conditions can be very sensitive and can be used to differentiate the causative virus. RT-PCR can either be performed on blood or CSF depending on the specific disease and is very valuable in combination with serology especially since early in disease serology may be negative.

Specific treatment

Treatment for most FV diseases is supportive. There is some evidence supporting the use of hyperimmunoglobulin for WN if administered early during the disease course.

Prevention

For mosquito-borne diseases control measures such as removal of containers that can act as breeding places and the prevention of bites by wearing long sleeved clothes and using mosquito nets and mosquito repellents are important.

Vaccines are available for YF, which is highly effective. A vaccine for JE is available and used in some endemic countries. Research on dengue virus vaccines is a priority.

Togaviruses

Table 1 **Classification**

Family – Togaviridae	
Genus	***Species***
Rubivirus	Rubella virus
Alphavirus	Chikungunya virus
	Sindbis virus
	Semliki Forest virus
	Ross River virus
	Western equine encephalitis virus
	Eastern equine encephalitis virus
	Venezuelan equine encephalitis virus

Table 2 **Structure and replication**

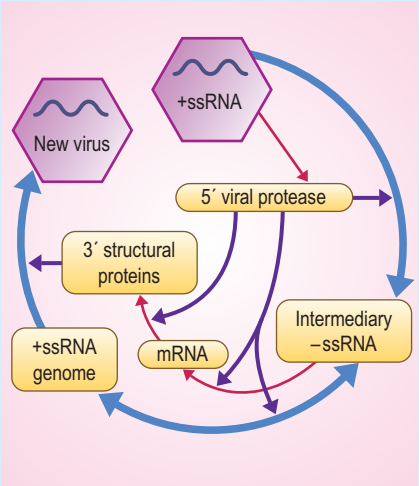
Structure	Replication
Positive sense single-stranded RNA Genome ~11–12 nucleotides long (rubella: 9800) Pleomorphic appearance Alphaviruses: 60–70 nm diameter Rubella: 51–65 nm diameter, with 6 nm surface projections (peplomers) that cause haemagglutination at 4 °C	

Fig. 2 **Replication cycle of togaviruses.**

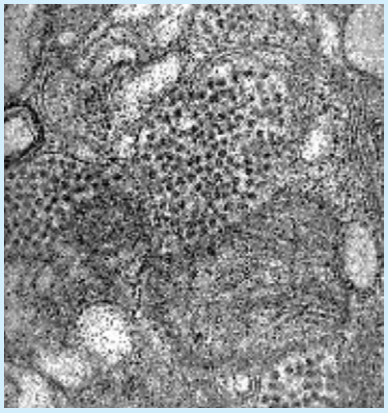


Fig. 1 **Alphavirus.** (Photo courtesy of CDC/Fred Murphy/Sylvia Whitfield.)

Rubivirus – Rubella

Epidemiology

Rubella occurs worldwide and humans are the only host. School children and young adults are the most affected, and infection is of significance in pregnant women. Occasional epidemics occur, but are not the rule, although local incidence increases every 3–4 years. Due to vaccination, rubella is rare in developed countries.

Pathogenesis

Post-natal infection

Rubella is spread by droplets from the respiratory tract. Primary replication occurs in the respiratory tract's epithelial cells, after which the virus spreads to the lymph nodes via the blood. Secondary replication occurs and the virus spreads to the skin, respiratory tract and other organs via the blood. Spread to the joints can cause arthritis, which is also thought to be partly as a result of immune-complex formation and may be influenced by hormonal factors, as post-pubertal women are the most affected. Rubella post-infectious encephalitis (1 in 5000–10 000 cases) may occur within 1 week of the rash onset and is believed to be immune-mediated, as are Guillain-Barré syndrome and thrombocytopenia, which are rare with rubella infection.

In-utero infection

The virus doesn't cause cell death or cytopathic effect, but inhibits growth and cell division by means of a viral antimitotic factor. If infection is during the period of organogenesis the fetus can develop severe organ malformations. Virus is shed for a prolonged period after birth, especially after first trimester infections.

Clinical picture

Post-natal infection

The incubation period is 14–20 days and 25–50% of infections are subclinical or mild enough to avoid diagnosis. Symptoms (see Fig. 4) start with malaise and fever, followed by lymphadenopathy, especially post-auricular. On day 3 of illness, the maculopapular rash (Fig. 3) appears simultaneously on the face, trunk and limbs. About 3 days later, the rash fades and generalised lymphadenopathy develops, and the patient usually recovers in the next 1–2 days. The patient is infectious during the period of 7 days prior to the rash until 7 days after the rash appears.

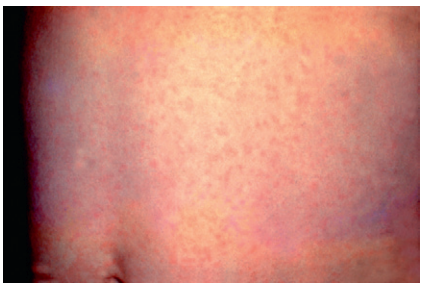


Fig. 3 **Rash caused by Rubella.** (Photo courtesy of CDC.)

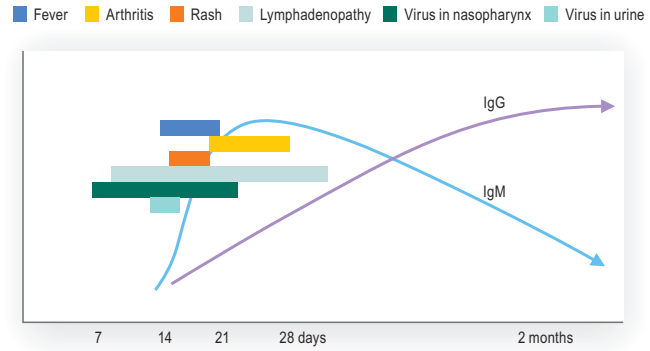


Fig. 4 **Timing and duration of rubella symptoms and signs in the context of viraemia and antibody production.**

The most common complication is arthritis, especially in post-pubertal women, usually of the hands, and sometimes other joints. It seldom lasts longer than 1 month and usually only several days.

Congenital infection

The most important aspects of congenital infection are defects of the central nervous system (microcephally, psychomotor retardation, behavioural disorders), cardiovascular system (septum defects, transposition of vessels), deafness, blindness and congenital cataracts (Fig. 5). For more details, see the chapter on congenital infections.

STOP THINK

- What other viral infections are problematic during pregnancy?
- How would you differentiate between the different childhood exanthems?

Fig. 5 **Cataracts in congenital rubella.** (Photo courtesy of CDC.)

Virological diagnosis

Serology:

Testing for immunity – IgG

Current/recent infection – IgM

If IgM and IgG are both positive, an avidity test can determine if the infection is primary or a re-infection – important in diagnosing rubella infection in pregnancy, if no previous sample is available for comparison.
IgM is usually detectable within a few days after onset of the rash, while IgG appears about a week later.

Isolation: rubella can be cultured on cells, using respiratory samples and urine, as well as amniotic fluid. In congenital infections, isolation should be done as early as possible after birth, to avoid detecting an early neonatal infection.

Rubella does not cause cytopathic effect, but inhibits the growth of other viruses, such as Echovirus 11, which can, therefore, be used to indicate the presence or absence of rubella infection in the cells.

Molecular: polymerase chain reaction (PCR) is ideal for amniotic fluid, and blood if taken at the correct time (between days 7 and 14 after infection).

Specific treatment

No specific treatment is available for rubella infection. After infection with non-vaccine rubella during pregnancy, termination can be considered, or intravenous rubella antibodies for those who choose against termination.

Prevention

The live-attenuated vaccine is the key to prevention of rubella and its consequences. It is commonly given as a combination vaccine with measles and mumps (MMR). It provides long-lasting immunity, is very effective and 95% of vaccinees develop an immune response within a month. Side effects include a rash, arthritis and lymphadenopathy. Pregnancy is a contraindication for receiving the vaccine, although serious effects have not been seen. Women should avoid falling pregnant within a month of vaccination.

Alphaviruses

Epidemiology

Alphaviruses are arboviruses, i.e. they are transmitted to humans via an arthropod vector, a mosquito in the case of alphaviruses (see Fig. 3, Chapter 5). The most well-known are Chikungunya, Sindbis, Semliki Forest and Ross River viruses. Chikungunya is spread by *Aedes* and *Culex* mosquitoes in Africa and Asia; Sindbis by *Culex* mosquitoes in Europe, Africa, Asia and Australia; Semliki Forest virus by *Aedes* mosquitoes in Africa and Asia; and Ross River virus by *Aedes* and *Culex* mosquitoes in Australia.

Pathogenesis

The pathogenesis is not completely understood, but is believed to be a combination of both viral infection and immune-mediated effects.

Clinical picture

The classic picture is that of fever, rash, headache, malaise and myalgia, with rash and arthralgia or encephalitis. Encephalitis is more common in the new world alphaviruses such as eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV) and Venezuelan equine encephalitis virus (VEEV), while the rash and arthralgia are more prominent in the old world alphaviruses. Sindbis infection is relatively common, but clinical disease is rare, as is clinical disease with Ross River virus. Chikungunya, which means 'that which bends up', is a debilitating arthritic disease that has been associated with outbreaks in areas where mosquito control has failed. The new world alphaviruses are generally found in North and South America. Haemorrhagic symptoms are rare, but have been reported, especially with old world viruses.

Diagnosis

Serology: IgM, IgG seroconversion, rising IgG titres,
Isolation:

Culture from blood, CSF, or synovial fluid

Demonstration of viral antigen in tissue

Cultured in suckling mice, mosquitoes or vero cells

Best within 48 hours of onset of illness

Molecular: polymerase chain reaction (PCR) on blood, CSF, synovial fluid or tissue.

Specific treatment

No specific treatment is available. Treatment is supportive, providing symptomatic relief.

Prevention

Vector control and mosquito bite prevention.

Recombinant alphaviruses

Alphaviruses such as VEEV and Sindbis are being investigated as vectors for genes that code for proteins useful in vaccines.

Key points

- Rubella is a common disease of childhood.
- Congenital abnormalities can result due to rubella infection during pregnancy.
- Rubella can be prevented with a safe, live attenuated vaccine.
- Alphaviruses are used as a vector in the design of various vaccines.

Hepatitis D virus

Introduction

Hepatitis D (HDV) is known as a subviral agent or defective RNA virus, that is related more closely to plant viroids than to human pathogens. It requires the help of another virus, hepatitis B virus (HBV), to transmit itself and establish infection. HDV is coated with HBsAg, which is necessary for entry and release from hepatocytes. Two major modes of infection with HDV are known. *Co-infection* with HDV occurs in an individual when he/she acquires both viral infections (HBV and HDV) simultaneously. This generally results in a more severe form of acute hepatitis than infection with HBV alone. An individual may be *superinfected* with HDV, when he/she already has an underlying chronic HBV infection. Superinfection may accelerate chronic liver disease in affected individuals and carries a higher risk for the development of fulminant hepatitis.

Structure

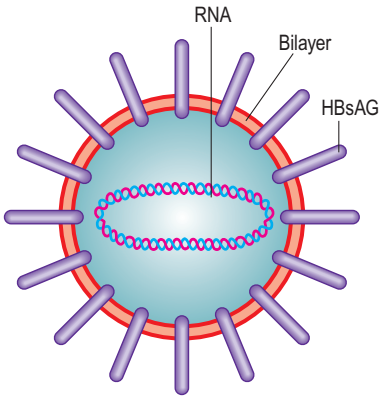


Fig. 1 **Hepatitis D virus (HDV) structure.**

Replication

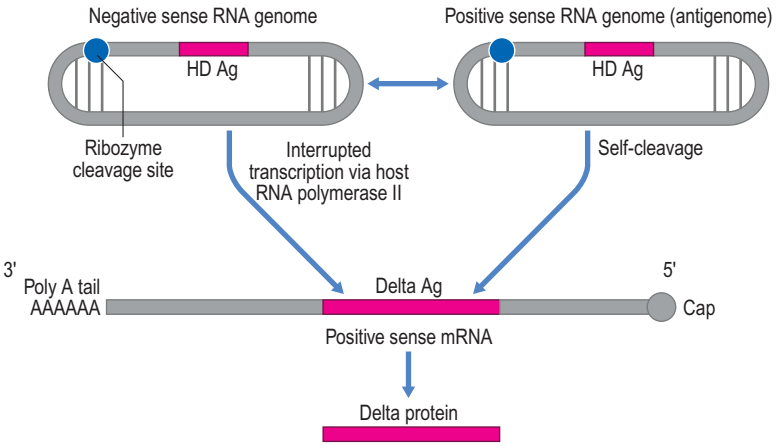


Fig. 2 **Hepatitus D virus (HDV) replication.**

Epidemiology

HDV was discovered in the mid-1970s when a group of HBV-positive patients with severe hepatitis was investigated. Today, more than 15 million of the 350 million carriers of HBV worldwide have serological evidence of exposure to HDV. The global distribution of HDV reflects the worldwide prevalence of hepatitis B. HDV is currently subdivided in eight genotypes. The infection is prevalent in South America, the Middle East, Japan, Taiwan, southern Europe and in parts of Africa. The routes of transmission and risk groups for HDV are similar to those of HBV, of which parenteral spread in intravenous drug users is especially important. Evidence for sexual transmission also exists.

Pathogenesis

The pathogenesis of HDV infection to date still remains largely unknown. The HDV receptor on hepatocytes remains unidentified, but is believed to be the same receptor as that of HBV. The delta antigen is not believed to be directly cytotoxic. Data suggests that the pathogenesis may be partly immunologically-mediated. HDV may also interfere with HBV replication, especially in the case of superinfection.

Clinical picture

HDV co-infection or superinfection can result in acute, chronic or fulminant hepatitis in conjunction with HBV. HDV tends to aggravate the underlying HBV infection. In HDV co-infection, the course of HDV infection is generally determined by the host response to HBV. In 95% of adults, HBV and HDV clearance result.

HDV superinfection of a patient with chronic HBV results in chronic HDV infection in most patients. HDV superinfection can also present as acute hepatitis in a previously undiagnosed HBsAg carrier. HDV superinfection generally results in more severe hepatitis and fibrosis, more rapid progression to cirrhosis and an increased rate of hepatic decompensation and death, than infection with HBV alone.

Table 1 **Markers for laboratory diagnosis of hepatitis D virus (HDV) co-infection and HDV superinfection**

	Markers for HBV infection				Markers for HDV infection			
	HBsAg	HBeAg	HBV DNA	Anti –HBc IgM	HD Ag	HDV RNA	Anti – HD IgM	Anti–HD IgG
co-infection	+	mostly–	+	+	+	+	+	+
Superinfection	+*	+*	+*	–	+	+	+	+

*, Markers of HBV replication may be suppressed by HDV superinfection

Laboratory diagnosis

Co- and superinfection with HDV have been shown to suppress the replication of HBV in patients. Every patient who is HBsAg-positive should be tested for anti-HDV IgG antibodies. Acute HDV infection can be diagnosed by the presence of anti-HDV IgM antibodies and/or HDV RNA. Serial measurement of HDV RNA levels are recommended to assess the response to antiviral therapy. HDV genotyping, although currently only available in specialist centres, is becoming a useful test in predicting adverse outcomes and response to antiviral therapy in certain high-risk genotypes (e.g. HDV genotype 1). Infection with HCV and HIV need exclusion, as co-infection with these viruses are common. Patients with positive HDV RNA should receive a liver biopsy to assess the severity of liver disease. Please see Table 1 and Fig. 3.

Treatment

The ideal endpoint of any treatment of HDV includes clearance of both viruses (HDV and HBV). Treatment options include pegylated interferon alone or in combination with tenofovir and emtricitabine. Interferon-alpha therapy for 6–12 months has shown considerable effect in the treatment of chronic delta hepatitis. It may repress HDV infection as well as decrease the biochemical markers of hepatic disease. Viral RNA has been shown to disappear from serum in approximately half of all treated cases. Many patients unfortunately experience relapses once treatment is stopped. HDV genotype 1 is associated with a decreased response to pegylated interferon. Liver transplantation is the only available option for patients with end-stage liver disease and should be considered for patients with acute liver failure who fulfill poor prognostic criteria.

Prevention

Immunisation against hepatitis B prevents HDV infection. General practices such as changing to disposable needles and syringes in intravenous drug-users and improvement of socio-economic conditions may prevent infection with HDV.

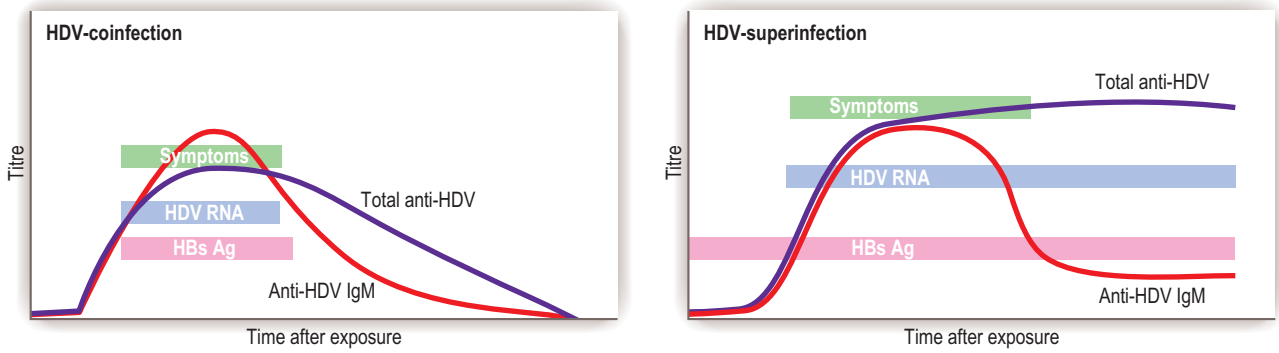


Fig. 3 **Serological course of hepatitis D virus (HDV) co-infection and HDV superinfection.**

Key points

- Hepatitis D is a defective virus or subviral agent that requires the help of another virus, hepatitis B, to infect hepatocytes.
- HDV is surrounded by an HBsAg envelope.
- Two modes of HDV infection occur. In HDV co-infection, HDV and HBV are acquired simultaneously. In HDV superinfection, HDV infects a host that is already chronically infected with HBV.
- HDV infection can aggravate existing liver disease caused by HBV and is an important cause of fulminant hepatitis.
- Liver transplantation and interferon alpha are available treatment modalities.
- Immunisation against hepatitis B can prevent HDV infection.

Prion diseases

Introduction and classification

The word 'prion' is derived from 'proteinaceous infectious particle'. The term was used in 1982 by Stanley B. Prusiner to describe the aetiology of a number of neurodegenerative conditions in mammals usually characterised by spongiform changes in the brain, which are called transmissible spongiform encephalopathies (TSEs) or prion diseases. These diseases are due to a conformational change in a normal human protein called PrP^{c} to a protease-resistant isoform, PrP^{Sc}, which accumulates in the CNS. Three epidemiological patterns are described: PrP^{Sc} arises spontaneously at a very low rate by conversion of normal PrP^{c} or by somatic mutation of the PrP gene in *sporadic disease*; rare germline mutations in the PrP-gene render PrP^{c} vulnerable to conformational change in *familial disease* and, lastly, when transmitted PrP^{Sc} interact with native PrP^{c} to induce conformational change to PrP^{Sc} it results in *transmitted disease*. The classification and aetiology of human prion diseases are shown in Table 1. Non-human TSEs include bovine spongiform encephalopathy (BSE), scrapie of sheep and goats, and chronic wasting disease of deer and elk.

Pathogenesis

Evidence that TSEs are caused by an agent that consists of protein-only and not nucleic acid was obtained from experiments that showed that they are resistant to procedures that would inactivate nucleic acid but are susceptible to long-term protease treatment; also PrP-gene knock-out mice that do not have the gene for PrP are not susceptible to prion disease.

Prion disease arises when abnormally folded prion protein, PrP^{Sc}, which is generally non-soluble and protease resistant, accumulates in the brains of affected individuals. The PrP-gene is on chromosome 20 in humans. PrP^{c} is expressed in neurons and may have a function in the binding of copper. Forty per cent of native PrP^{c} consists of α-helix and only 3% of β-sheet; it has a half-life of about 5-hours and is rapidly destroyed by proteases, whereas PrP^{Sc} consists of 43% β-sheet and only 30% α-helix; it is protease resistant and accumulates in cells. The first step and critical event, preceding prion protein accumulation is the formation of PrP^{Sc}. PrP^{Sc} can arise endogenously by sporadic somatic mutation of the PrP-gene or spontaneous conformation change of normal PrP^{c}. When an individual has certain germline mutations of the PrP-gene, PrP^{c} is unstable and can more easily convert to PrP^{Sc}. Therefore, the age of onset of familial disease is earlier than for sporadic disease. Once PrP^{Sc} is formed it acts as a catalyst and interacts with PrP^{c} further converting that to PrP^{Sc}. The result is a chain reaction which can be referred to as 'prion replication'. The formation and 'replication' of prion protein is illustrated in Fig. 1.

Epidemiology

The incidence of sporadic Creutzfeldt–Jakob disease (sCJD) is about 1–2 per 1 000 000 per year. The incidence of sCJD is quite constant worldwide and there is no significant gender difference. Eighty-five per cent of CJD cases are

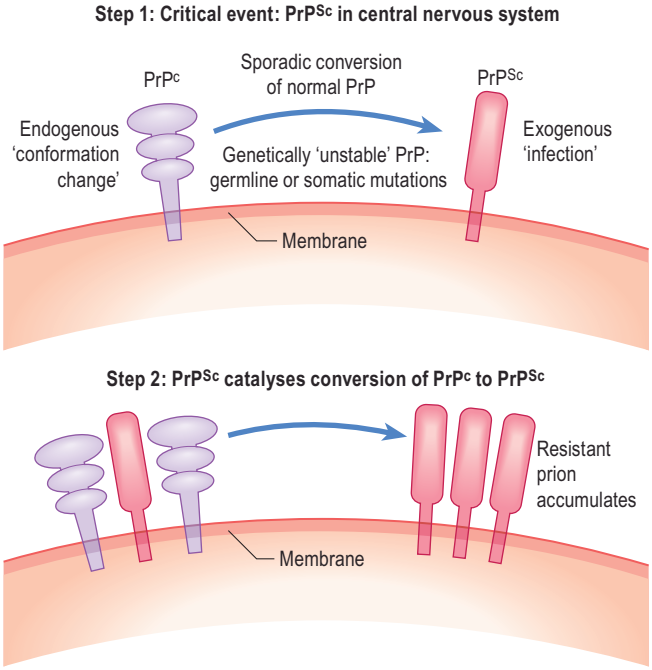


Fig. 1 **The formation and 'replication' of prion protein.**

Table 1 **Classification and aetiology of human prion diseases**

Prion disease	Epidemiological classification	Aetiology
Creutzfeldt-Jakob disease (CJD)	Sporadic CJD (sCJD)	Spontaneous conformational change in prion protein or somatic mutation of the gene
	Familial CJD (fCJD)	Germline mutations such as E200K and D178N render proteins susceptible to conformational change
	Iatrogenic CJD (iCJD)	Corneal and dura mater transplants, invasive EEG electrodes, other neurosurgical procedures and human growth hormone administration resulted in prion transmission
	Variant CJD (vCJD)	Consumption of beef products contaminated with bovine spongiform encephalopathy (BSE)
Gerstmann-Sträussler-Scheinker disease (GSS)	Familial GSS	Germline mutations such as P102L and G131V are associated with a typical presentation. Other mutations may lead to a different phenotype
Fatal insomnia	Fatal familial insomnia (FFI)	Germline mutation D178N found in association with 129M polymorphism on the same gene
	Fatal sporadic insomnia (FSI)	Spontaneous conformational change in prion protein or somatic mutation of the gene
Kuru	Human-to-human transmitted	Prion protein historically transmitted by ritual cannibalism among the South Fore people of Papua New Guinea

sporadic and 5–15% are due to inherited germline mutations. Inherited mutations are also responsible for Gerstmann Sträussler syndrome (GSS) and fatal familial insomnia (FFI), although a sporadic form, fatal sporadic insomnia (FSI), has also been reported. These conditions are rarer than CJD.

Variant CJD (vCJD) was first reported in 1996 in the United Kingdom (UK) when it was realized that bovine spongiform encephalopathy (BSE) may have been transmitted to humans. The clinical and pathological features of vCJD differed significantly from sCJD. Epidemiological evidence indicates both a temporal (vCJD epidemic followed BSE epizootic with about 8–10 years delay due to the prolonged incubation period) and geographic link: BSE was predominantly found in the UK. By the end of 2011, 176 definite or probable cases of vCJD have been reported in the UK, 25 in France, that imported meat from the UK, and 3 in the USA. Experimental inoculation of macaque monkeys with BSE resulted in similar pathology to vCJD in humans which also supported this hypothesis. Homozygosity of methionine at position 129 of the PrP-gene was found in all vCJD cases studied.

Kuru, a disease which was found in the South Fore people of Papua New Guinea was especially prevalent in women and children since it was them, primarily, who ate the brain of diseased relatives during ritual cannibalism. Since cannibalism ceased in the 1950s the incidence of kuru declined. Even in kuru genetics played a role since homozygosity at position 129 predisposed to increased risk and early onset of kuru.

Clinical manifestations

The classical triad of sCJD are dementia (which may start as forgetfulness and progress to full dementia), ataxia (loss of balance), myoclonus (muscle jerks) and characteristic periodic bursts on the electroencephalogram (EEG). The mean age of onset is 60 years. A prodrome of fatigue, headache, sleep disturbance, vertigo and behavioural changes may precede dementia and ataxia. Myoclonic jerks usually manifests later. In the final stage patients become mute, bed-ridden and incontinent. The average duration of disease is 4–5 months and death typically results from respiratory complications or sepsis. In contrast, the onset of familial CJD (fCJD) is usually between 40 and 60 years, but may occur as early as 25 years and the reported duration of illness (1–5 years) is longer than for sCJD.

vCJD differs from sCJD in a number of characteristics. The mean age of onset of vCJD is 27 years. Behavioural changes are prominent at disease onset, with some patients reporting unpleasant and painful sensation, and patients often being referred for psychiatric care since dementia and other neurological symptoms usually manifest only later during disease course. The average duration of illness is 14 months. On neuropathological investigation sCJD and fCJD are characterized by generalized grey matter vacuolation and gliosis, whereas vCJD is characterized by PrP plaques surrounded by vacuolation.

In case of GSS, patients present with ataxia and dysarthria, pyramidal and extrapyramidal symptoms with dementia following late. Neuropathological findings are that there is no or minimal spongiosis, but extensive PrP plaques and neurofibrillary tangles.

People with FFI present with untreatable insomnia followed by dysautonomias, ataxia and pyramidal and extrapyramidal symptoms. Dysautonomia includes changes in blood pressure, temperature, heart rate and respiratory rate. Neuropathological findings include gliosis of the thalamus, inferior olive nuclei and cerebellum.

Diagnosis

Preliminary diagnosis is based on the constellation of clinical symptoms and signs, the exclusion of other conditions, a positive EEG, family history and identification of a germline mutation in familial cases. Neuropathological diagnosis is by brain biopsy or autopsy with the performance of immunohistochemistry and immunoblot. The ultimate diagnosis, however, is by inoculating brain material into a susceptible host animal species such as transgenic mice and then to observe the clinical and pathological changes. The detection of the 14-3-3 protein in the CSF and neurospecific enolase is not specific enough to make a diagnosis. An in-vitro method called 'cyclic amplification of protein misfolding', which amplifies PrP^{Sc} to ease diagnosis, has also been developed.

Specific treatment

No clinically proven treatment is available. However, many experimental agents have been evaluated in-vitro. These are not targeted at curing prion disease, but at delaying the progression of disease. Agents such as tannic acid and pentosan polysulphate inhibit PrP^{Sc} in-vitro. A novel strategy that shows promise in in-vitro experiments is the use of small interfering RNA (siRNA), which induces the degrading of PrP mRNA resulting in a reduction in PrP^{C} production.

Prevention

Since prion diseases are incurable the emphasis should remain on prevention. The use of mammalian products as protein and mineral supplements for livestock should be discouraged following epidemiological and experimental evidence that this practice was linked to the BSE epidemic in the UK. The culling and incineration of BSE-infected cattle herds has been effective in reducing the incidence of vCJD. Iatrogenic transmission can be prevented by the effective screening of potential organ or tissue donors (especially in cases of cornea and dura-mater transplants) for symptoms that could be compatible with TSEs, a family history of TSEs, or history of potential exposure, combined with stringent infection control measures. Disposable equipment should preferably be used for diagnostic procedures in cases of potential prion disease. Surfaces should be disinfected with sodium hydroxide (NaOH). Since PrP^{Sc} is highly resistant sterilization requires special measures such as emersion in 1 molar NaOH and autoclaving at 121°C for 30 minutes or autoclaving at 134°C for 18 minutes. Careful removal and incinerations of waste should also occur. Since cases of CJD after blood transfusion have been documented, blood donors should also be screened for symptoms, family history or exposure history. Special prion reduction filters have recently been developed which may in future help to safeguard blood.

Neurological disease with a viral aetiology

Important terms and definitions

Neurotropism: having a preference for neural cells.

Neurovirulence: causing disease in the nervous system.

Pathogenesis of central nervous disease

Viruses enter the CNS transported by nerve tissue (Fig. 1), called neurogenic spread (rabies and probably some cases of herpes simplex virus (HSV) encephalitis) or via the blood stream (haematogenous spread) with transfer across the blood–brain barrier by infection of migrating leucocytes (Fig. 2), endothelial cells (Fig. 3) or by crossing the pores of the choroid plexus (Fig. 4). Infection of the endothelial cells with accompanying vasculitis is common in arboviral encephalitis. Viruses causing encephalitis also infect neurons and glial cells. Infection of anterior motor neurons in the spinal cord results in acute flaccid paralysis associated with polio and other enteroviruses. Direct infection of oligodendrocytes results in progressive multifocal leucoencephalopathy caused by JC polyoma virus. Auto immune destruction of myelin following infections or vaccinations can either lead to acute inflammatory demyelinating polyradiculoneuropathy (Guillain–Barré syndrome) or acute disseminated myeloencephalopathy (ADEM) in the nerve roots or white matter of the brain respectively.

Spectrum of disease

Epidemic aseptic meningitis

Enteroviruses (coxsackie, echoviruses, entero 70, 71) are the most common cause of meningitis in children, worldwide. Outbreaks usually occur in summer months. Spontaneous recovery is the norm. More severe disease occurs in neonates associated with systemic disease. Rarely enteroviruses may also cause encephalitis.

Mumps virus meningitis

This occurs in approximately 50% of cases of mumps (15% is symptomatic) but can also occur in absence of classic mumps parotitis in 50% of cases. Meningo-encephalitis and hearing loss are rare complications.

Sporadic meningitis

Genital herpes simplex

Benign meningitis is common with primary and recurrent genital herpes.

Other herpes viruses

Varicella zoster virus (VZV) can reactivate to cause meningitis in the absence of a rash. VZV is a cause of diffuse encephalitis in non-immunocompromised adults. Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpes virus 6 and 7 (HHV-6 and HHV-7) are also associated with meningitis or meningoencephalitis.

Adenovirus

Neurological disease associated with adenovirus is usually aseptic meningitis, but serotype 7 can cause severe meningoencephalitis.

Lymphocytic choriomeningitis virus

A chronic meningitis transmitted by the common house mouse.

Acute HIV infection

Meningitis occurs in up to 17% of cases of acute HIV infection and is associated with rapid progression of disease.

Herpes simplex virus encephalitis

This is a destructive encephalitis, usually unilateral, which involves the temporoparietal lobes. Except in infants where HSV-2 is predominant, it is usually primary or recurrent HSV-1 infection. In neonates and immunosuppressed individuals HSV can cause diffuse encephalitis.

In the absence of treatment mortality is about 70–90%. Early anti-herpes virus therapy (acyclovir) reduces mortality to less than 20% with almost 40% fully recovering.

Arboviral meningitis and encephalitis

These viruses cause regional seasonal meningitis and encephalitis and are transmitted by arthropod vectors (mosquitoes or ticks). These include flaviviruses (e.g. Japanese encephalitis virus, West Nile virus and tick-borne encephalitis viruses), bunyaviruses (e.g. Rift Valley fever virus) and alphaviruses (e.g. Ross River virus).

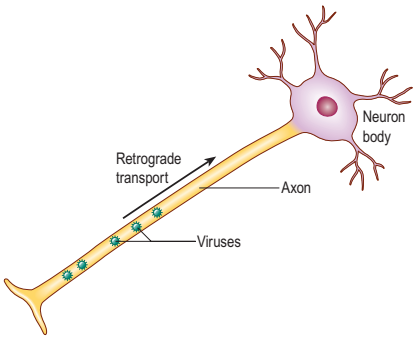


Fig. 1 **Neurogenic spread (axonal transport).**

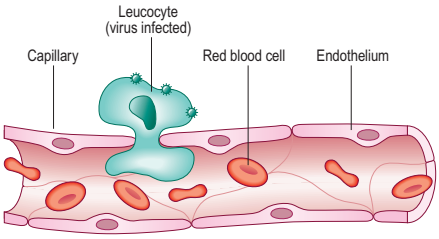


Fig. 2 **Leucocyte migration.**

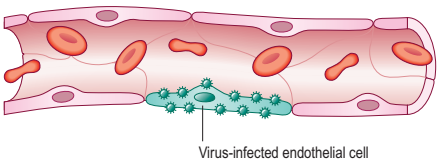


Fig. 3 **Endothelial infection.**

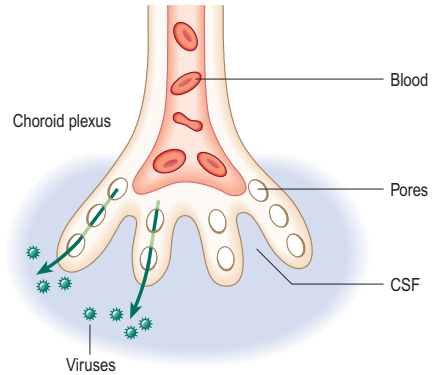


Fig. 4 **Virus entry into CNS.**

Post-viral encephalitis

Acute disseminated encephalomyelitis (ADEM) is an immune-mediated encephalitis that can follow various viral infections: measles virus, VZV, EBV, CMV, HSV, rubella virus, hepatitis A virus and coxsackie virus.

Acute flaccid paralysis

Polioviruses 1 and 3 still cause cases of acute flaccid paralysis (AFP) in countries where it remains endemic. In rare cases it is caused by enterovirus 70, 71 or coxsackie virus A7.

Central nervous system disease in immunosuppressed patients

Progressive multifocal leucoencephalopathy (PML) is characterised by patchy demyelination of the white matter of the brain. Patients present with sensory deficits and motor weakness. PML is common in patients with AIDS and/or other causes of immunosuppression.

Herpesviruses, such as HSV, VZV, CMV, EBV, HHV-6 and HHV-7, can all cause encephalitis in severely immunocompromised patients (bone marrow transplants). Adenovirus is a rare cause of fatal encephalitis in transplant patients.

HIV-associated dementia is seen where there is infection of neuroglia and the toxic effect of some HIV proteins are the likely causes. It usually occurs late in the course of HIV disease.

Measles inclusion body encephalitis (characterised by its histological appearance) is a rare complication of measles infection in immunocompromised patients.

Progressive encephalitides

Subacute sclerosing panencephalitis (SSPE) is a rare disease following 1–10 years after measles virus infection. It is characterised by progressive dementia, refractory convulsions and has a characteristic EEG pattern. It is invariably fatal. Very rarely a disease called progressive rubella panencephalitis can also occur after rubella infection.

Since they do not have viral aetiology, prion diseases are discussed in another section.

Zoonotic encephalitis

These are encephalitides from contact with other mammals. Rabies and rabies-like viruses (genus Lyssavirus) are transmitted by the saliva of infected carnivores and bats. Hendra and Nipah viruses, which are paramyxoviruses of bats, caused outbreaks in humans by first infecting domestic animals. Herpes B, a simian herpes virus can cause lethal encephalitis in exposed animal handlers and laboratory workers in the absence of timely acyclovir post exposure prophylaxis.

Acute inflammatory demyelinating polyradiculoneuropathy or Guillain–Barré syndrome

This is a rare auto-immune inflammatory condition affecting nerve roots that could be triggered by various infections (including many viruses) and vaccinations, and is clinically characterised by ascending paralysis.

Diagnosis

Patient history and clinical signs guide the special investigations in the evaluation of these patients.

Meningitis

Viral meningitis can usually be differentiated from bacterial meningitis: the CSF is usually clear on visual inspection and has a normal glucose, there is predominance of lymphocytes rather than neutrophils and Gram stain shows no bacteria with a negative bacterial culture. Reverse transcription polymerase chain reaction (RT-PCR) is used to diagnose enteroviral meningitis on CSF specimens.

Encephalitis

EEG can be valuable to assist diagnosis and CT scan or MRI showing unilateral temperoparietal necrosis with surrounding oedema is highly suggestive of HSV infection; however, the CT scan may initially be normal. A PCR for HSV-DNA on CSF can confirm the diagnosis. Arbovirus encephalitides are usually diagnosed by detecting specific antibodies in serum or CSF or by RT-PCR on CSF.

The CT scan and MRI are very characteristic of PML. This can be confirmed with a PCR for JC virus on the CSF.

PCRs for HSV, CMV, EBV, HHV-6, HHV-7 and JC virus are used to screen immunosuppressed patients with encephalitis.

Treatment

Treatment is available for herpes simplex encephalitis. Empiric therapy with anti-herpes virus drugs such as acyclovir is necessary when the diagnosis is suspected. PML in HIV patients and HIV dementia respond somewhat to HAART, but should rather be prevented by timely use of HAART. For most other CNS diseases there is no specific treatment and management is symptomatic and supportive.

Prevention

Vaccination and post-exposure prophylaxis for rabies are discussed in another section. Vaccination for common childhood diseases such as measles, rubella and varicella dramatically reduce the risk of the respective CNS complications.

Vector control is important in preventing arboviral encephalitis. Vaccines are also available for Japanese encephalitis and some of the other arboviruses. Animal handlers exposed to herpes B virus should immediately use acyclovir post-exposure prophylaxis.

- It is summer and the patient is a child with meningitis and clear CSF, normal glucose and normal neutrophils; what would be the most likely diagnosis?
- Why should empiric aciclovir treatment be started in a comatose patient with focal neurological signs?

Key points

- Enteroviruses are the most common cause of aseptic meningitis in children.
- Herpes simplex virus (HSV) encephalitis usually involves the one temporal or parietal lobe in immune competent adults and children.
- Early anti-herpes virus therapy has a dramatic effect on the outcome of HSV encephalitis and should, therefore, be empirically initiated on suspicion of HSV encephalitis.

Gastrointestinal illness

Introduction

Viruses are the most common cause of gastroenteritis worldwide. Around about 4–6 million people die from diarrhoeal disease every year. Human rotaviruses (RVs), caliciviruses, astroviruses HAsV and adenoviruses are most commonly associated with gastroenteritis, although many other viral diseases may be complicated by gastrointestinal symptoms. Even outbreaks of severe acute respiratory syndrome (SARS), coronavirus, H5N1 avian influenza and Ebola were associated with gastroenteritis.

RV is the most common worldwide cause of infantile diarrhoea and the most common cause of hospitalisation due to infantile gastroenteritis in industrialised countries. Other common viral causes are human astroviruses, human enteric adenoviruses and caliciviruses. Caliciviruses (genus norovirus (NV)) are the most common cause of gastroenteritis outbreaks in developed countries.

Rotavirus

Properties

RVs are double-shelled, naked, round particles with a characteristic appearance on electron microscopy (EM), hence the name rotavirus (rota means wheel in Latin). These viruses are about 70 nm in diameter and contain 11 segments of double-stranded RNA. RVs are very resistant to desiccation and can survive in the environment or in faeces for more than 7 months.

Epidemiology

Rotavirus outbreaks are more common in winter in temperate regions. RVs spread by faecal-oral and possibly respiratory route. RVs cause about 50% of human infantile gastroenteritis cases. Outbreaks typically occur in crèches and hospitals.

There are many different RVs serotypes allowing for repeated infections in humans, although there is some immunological cross-protection. The first rotavirus infection, therefore, tends to be the most severe. RVs are responsible for 600 000–1 million deaths per year and are the leading cause of hospitalisation due to diarrhoea.

Pathogenesis

The incubation period is about 1–4 days. RV infects enterocytes resulting in villous atrophy of duodenum and upper ileum. Enterocytes need to regenerate for individuals to have clinical recovery. Since villous atrophy (Fig. 1) leads to loss of brush border enzymes rotavirus infection is often followed by prolonged diarrhoea and lactose intolerance. A viral protein NSP4 also acts as enterotoxin causing a secretory component to diarrhoea. RV can also activate the intestinal nervous system leading to an increase in secretion of fluid and electrolytes. Infection is not only restricted to enteric tract, but virus can be detected in the blood in some cases of infection, which may explain the several systemic symptoms.

Clinical features

Fever and vomiting is followed by severe watery diarrhoea of up to 20 stools per day. Disease usually occurs in children under the age of 2 years. Duration is from 3 to 9 days. RV infection is a common cause of severe dehydration. A temperature of above 39°C is a common finding. On the other hand infection may be asymptomatic, especially in individuals under 6 months or after 5 years of age. RV is also associated with systemic and respiratory disease in infants.

Human caliciviruses

Properties

These are icosahedral naked viral particles, about 35 nm in diameter with cup-like indentations. Calicici is derived from the Latin word 'Calix', which means cup. The viral genome is a single positive RNA strand of approximately 7.5 kb in size.

Epidemiology

Caliciviruses are the most common cause of sporadic acute gastrointestinal illness except in infants. Outbreaks occur all year round. There are two genera affecting humans: NV, which includes the most common causes of outbreaks, and sapovirus, usually associated with gastroenteritis in children. There are many different strains of NVs belonging to four genogroups. A single individual may suffer many NV infections in a lifetime. NVs have very low infectious doses and less than 10 virions could be enough to cause disease in a healthy adult.

Spread is faecal–oral and outbreaks from contaminated water and food sources are common, but are usually accompanied by further secondary person-to-person spread. Faeces and vomitus are infective and viruses may be aerosolised during projectile vomiting. Caliciviruses also survive in the environment on surfaces and contaminated fomites can play a role in outbreaks. The incubation period is usually 1–2 days, but may be as short as 12 hours. Outbreaks have been reported in nursing homes, military bases, school campuses and cruise ships. Attack rates are about 41% with the rest having asymptomatic infection.

Pathogenesis

Little is known about the pathogenesis of human caliciviruses. Genetic susceptibility and immunity to NVs are discussed in the calicivirus chapter.

Clinical features

Individuals present with nausea and vomiting that can be projectile, stomach pain and watery diarrhoea. Carriers

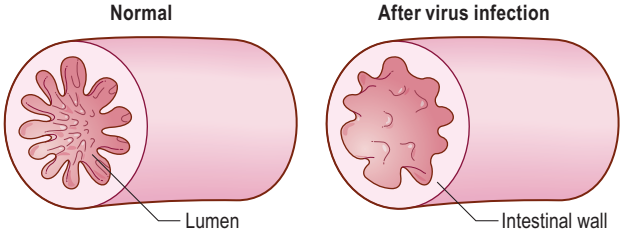


Fig. 1 **Villous atrophy.**

- A breastfeeding child presents with vomiting, watery diarrhoea, 10% dehydration and a fever of 40°C. Is this more likely to be viral or bacterial gastroenteritis? How would you manage the child?
- It has been found that despite strict hand washing and other standard precautions, nosocomial outbreaks of rotavirus still occur. Why would that be?
- How could the epidemiological pattern help you to distinguish a norovirus outbreak from bacterial food poisoning?

may be asymptomatic, but asymptomatic carriers are less infectious. The duration of illness usually is 1–3 days.

Human astroviruses

Properties

These are 30 nm naked icosahedral viruses. On EM they can appear as five or six pointed stars. Genomes are positive single-stranded RNA of about 7 kb in size.

Epidemiology

Human astroviruses (HAsVs) are a common cause of endemic diarrhoea. They are usually spread from person to person. Large common source outbreaks (food and waterborne) also occur. Outbreaks were reported in schools, geriatric care facilities, and adult and paediatric wards in hospitals. It is also associated with gastroenteritis in surfers who are exposed to contaminated sea water. During common source outbreaks secondary transmission can occur. Incubation is 1–2 days. HAsV can also cause diarrhoea in immunosuppressed patients.

Pathogenesis

Little is know about HAsV pathogenesis. (For more information see HAsV chapter.)

Clinical features

Diarrhoea is usually mild – lasting from 1 up to 14 days. (For more information see AV chapter.)

Human enteric adenoviruses (adenovirus 40 and 41)

Properties

Adenoviruses are naked capsids, about 80 nm in diameter. On electromicroscopy characteristic penton fibres are visible on apices. The genome is double-stranded linear DNA 36–38 kb in size.

Epidemiology

There is no clear seasonal peak incidence. Transmission is predominantly by person-to-person spread. Enteric adenoviruses are the second leading cause of hospitalisation for viral gastroenteritis in infants. The average incubation period is 2–3 days.

Pathogenesis

Except for the fact that enterocytes are infected, little is known about the pathogenesis of adenoviruses gastroenteritis.

Clinical features

Enteric adenoviruses cause a similar clinical syndrome to rotavirus infantile gastroenteritis, although diarrhoea predominates. Duration of symptoms is from 1 day up to 7 days. It tends to be less severe than rotavirus diarrhoea. Adenovirus diarrhoea is sometimes complicated by intussusception.

Laboratory diagnosis of viral diarrhoea

A stool sample is preferable above a rectal swab for diagnosis. Rapid diagnosis is available for RV and adenovirus 40 and 41, and can be performed either by latex agglutination tests or an enzyme immuno assay (EIA), which is generally the more sensitive method but needs the availability of a laboratory facility. Where EM is available it can be both a rapid and sensitive test for diagnosis of RV diarrhoea, since the virus is excreted in very high quantities. EM is also valuable for diagnosing HAsV and calicivirus diarrhoea, but not for adenovirus diarrhoea since it cannot distinguish between diarrhoea causing (adenovirus 40/41) and other adenoviruses excreted in stool. Molecular techniques such as reverse transcriptase polymerase chain reaction (RT-PCR) or PCR are becoming the diagnostic methods of choice since it is most sensitive and could genotype the strain, thus proving to be very valuable in investigating outbreaks.

Prevention of viral diarrhoea

General prevention includes sanitation and hygiene. In health-care settings hand-washing and disinfection of surfaces are important, although viruses can also spread by aerosol. Vaccines have not been available except for RV, for which the first registered RV vaccine (Rotashield, 1998), which was a human-rhesus reassortant vaccine was withdrawn due to an increase in the incidence of intussusseption in vaccinees. (New vaccines are discussed in the rotavirus chapter.)

Treatment of viral diarrhoea

No specific treatment exists for viral diarrhoea. Replacement of fluid and electrolytes are essential; either by oral rehydration or intravenous access in case of severe dehydration or frequent vomiting.

Key points

- Rotaviruses (RVs) are the most common cause of infantile gastroenteritis worldwide.
- RVs can cause high fever and severe dehydration and are the most common cause of hospitalisation due to infantile gastroenteritis in industrialised countries.
- New promising RV vaccines have recently become available.
- Noroviruses are the most common cause of gastroenteritis outbreaks in industrialised countries.
- A stool sample for molecular testing is very valuable to characterise and describe viral gastroenteritis outbreaks.

Respiratory viruses

Introduction

Respiratory tract infections are common, and a major cause of morbidity and mortality around the world. Viruses are responsible for about 90% of upper respiratory tract infections, and about 30% of lower respiratory tract infections. Each respiratory syndrome can be caused by a number of different viruses, and each individual virus can result in several different clinical presentations.

Several respiratory tract infections per year are common, due to the easy spread of a large variety or organisms via aerosol and droplet spread, as well as fomites (e.g. through contact with objects on which other people may have, for example, sneezed).

Spectrum of disease

Table 1 lists the common conditions, and associated viruses. For individual viruses see the relevant chapter.

Individual causative agents: epidemiology, pathogenesis and clinical picture

Influenza viruses

Influenza affects all ages and is a significant cause of respiratory disease worldwide. There are three types – influenza A, B and C. Avian influenza, a potential threat to humans, is a type of influenza A. Influenza C causes a much milder clinical picture than the other viruses, while influenza A has caused several pandemics, the most well-known being the Spanish influenza of 1918. Annual epidemics occur in the winter season, and are currently caused by influenza B and influenza A H1N1 and H3N2.

Table 1 **Common conditions and associated viruses**

Condition	Common viral causes	Other viruses
Rhinitis/common cold	Rhinoviruses Coronaviruses	Adenoviruses Enteroviruses Influenza viruses Human metapneumovirus Parainfluenza Respiratory syncytial virus (RSV)
Pharyngitis	Adenoviruses Enteroviruses – Coxsackie A Influenza viruses Parainfluenza	Herpesviruses – herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) Rhinoviruses
Otitis media	Adenoviruses Rhinoviruses	Coronaviruses Human metapneumovirus RSV
Laryngotracheobronchitis (croup)	Coronaviruses Parainfluenza RSV	Adenoviruses Influenza Herpes simplex Measles
Bronchiolitis	Parainfluenza RSV	Adenoviruses Coronaviruses Enteroviruses Human metapneumovirus Human bocavirus Rhinoviruses
Pneumonia	Adenovirus CMV Influenza Parainfluenza RSV	Human metapneumovirus Human bocavirus Coronaviruses Measles Varicella

Fig. 1 **Influenza vaccine pack.** (Photo courtesy of sanofi pasteur.)

The incubation period is 12–72 hours, followed by an abrupt onset of systemic symptoms. Infection is characterised by a cough, sore throat, fever and myalgia, although a wide range of presentations can occur, ranging from mild to severe with complications. Complications include secondary bacterial infections, viral pneumonia, myocarditis, myositis and Reye's syndrome.

Vaccination (see Fig. 1) is one of the most important means to control the annual epidemics. Available vaccines are usually subunit vaccines (killed) but live attenuated vaccines are available in some countries. The indications and contraindications for the subunit vaccine are shown in Table 2.

Antiviral drugs are available for the treatment of influenza and need to be started early in order to be effective. Amandatine and rimantadine, ion channel blockers, prevent influenza A from uncoating after it infects a cell. Oseltamivir and zanamivir are neuraminidase inhibitors that prevent release of new viruses from the cell surface. Resistance to these drugs is common.

Table 2 **Indications and contraindications for the influenza subunit vaccine**

Indications	Contraindications
Elderly – over 50 years	Egg allergy
Infants – 6 months–4 years	Acute severe febrile illness
Underlying medical conditions, such as diabetes, chronic lung or heart disease, obesity	Note: The indications for medical conditions, and the use in health-care workers exposed to these individuals with these conditions are considered to be contraindications for the use of the live attenuated vaccine.
Age 6 months–18 years on chronic aspirin therapy	
Women who will be pregnant during the influenza season	
Immunosuppressed persons (they need to be able to respond to the vaccination, however)	
Health-care workers, especially those working with those with other indications	
Workplace – avoidance of loss of productivity	
Personal desire to avoid influenza infection	

Parainfluenza viruses

There are four parainfluenza viruses, types 1–4, of which type 3 is the most common cause of the more severe disease. Parainfluenza 3 is seen throughout the year, with a peak in spring, while types 1 and 2 are seen in autumn.

The virus exhibits tropism for the ciliated epithelial cells lining larger airways. In infants infection usually presents as bronchiolitis, and in older children as croup. Pneumonia is rare in healthy children, but may be associated with underlying conditions such as prematurity or asthma. In adults, infection may present as bronchitis or rhinitis.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is one of the main causes of hospitalisation of children in the first year of life in most

STOP THINK

- What respiratory conditions are seen mainly in immunocompromised populations?
- Why will old specimens not produce optimal results?
- Why are children on chronic aspirin therapy given influenza vaccines?

parts of the world. By the age of 3 years most children have been infected.

The virus replicates in the respiratory mucosa, involving the lower respiratory tract. After an incubation period of 2–8 days, the clinical presentations include mild upper respiratory tract infection, otitis media, croup, pneumonia and bronchiolitis. Bronchiolitis presents with dyspnoea, tachypnoea, respiratory distress, wheezing and hyperinflation of the lung on X-ray. In premature infants and neonates, apnoea may be the only sign of infection. Symptoms usually resolve within a week, but if infants are sick for a longer period, they may require hospitalisation. In about 75% of those who develop RSV bronchiolitis, chronic wheezing can be present for a number of years. Infections in adults are less severe, although in the elderly and immunocompromised disease can be severe.

Treatment is usually supportive, with oxygenation, and bronchodilators and ventilation if necessary. Ribavirin can be used for treatment of severe cases. For prophylaxis, RSV immunoglobulin has been used, but palivizumab, a recombinant monoclonal antibody, has proved to be more effective. Table 3 gives a summary of the American Academy of Pediatrics guidelines for using palivizumab. Vaccines are not available, but are being investigated.

Table 3 **Summary of the American Academy of Pediatrics guidelines for using palivizumab**

Infants <24 months old with chronic lung disease who have required medical therapy in the last 6 months.
Infants <12 months old at the start of the respiratory syncytial virus (RSV) season, born before 28 weeks' gestation, and who have chronic lung disease.
Infants <6 months old at the start of the RSV season, and who were born before 32 weeks' gestation.
Infants <6 months old at the start of the RSV season, who were born between 32 and 35 weeks' gestation and have other risk factors, e.g. crowding at home, exposure to smokers, school-going siblings, one of twins.

Adenoviruses

Adenoviruses are believed to cause about 10% of respiratory illness worldwide, and can manifest as any of the most common respiratory presentations, although the common cold, pharyngitis, and otitis media are the most frequently seen. Pneumonia in young children is also of concern. No treatment exists, although ribavirin use has been reported. A vaccine for certain types is limited to use by military personnel and safety for children is of concern.

Rhinoviruses

Rhinoviruses are found worldwide, and over 100 serotypes are known, which means that symptomatic infection with different strains often occurs. Spread is usually via nasal secretions. Rhinoviruses are the most common cause of the common cold, but can also cause other respiratory syndromes, especially in children with asthma, cystic fibrosis or other chronic lung diseases.

Coronaviruses

Five human coronaviruses are known; four circulate worldwide and affect all age groups, and the fifth, the cause of SARS no longer circulates. See the chapter on coronaviruses for more detail.

Cytomegalovirus

Cytomegalovirus (CMV) is seen worldwide, amongst all ages. Pneumonitis is found mainly in immunocompromised adults and in premature and immunocompromised infants. Treatment is possible with gancyclovir, valgancyclovir, foscarnet and cidovofir.

Diagnosis of viral respiratory tract infections

Diagnosis is best made on samples taken within the first 48 hours of infection, when the viral load in the respiratory tract is high. For lower respiratory tract infections, bronchial lavage, bronchioalveolar lavage and tracheal aspirates are the best specimens, although the viruses can sometimes be isolated from upper respiratory samples, such as nasal or throat swabs or nasopharyngeal aspirates. Samples should be sent to the laboratory as soon as possible with viral transport medium and storage at 4°C to preserve live virus.

Serology: this is seldom used for purposes other than epidemiological surveillance.

Isolation: rapid tests on respiratory samples are available for some viruses, with RSV and influenza testing used the most.

Indirect immunofluorescence: on cells in respiratory samples this may also provide a rapid diagnosis.

Cell culture: this is an important means of diagnosis using, e.g. vero and HeLa cells, which support the growth of most respiratory viruses. Many respiratory viruses grow better at 33°C instead of 37°C, as this corresponds better with the temperature of the respiratory tract.

Molecular: polymerase chain reaction (PCR) is commonly used for metapneumovirus, bocavirus and the coronaviruses, but can be applied to all respiratory viruses. It is usually only available in certain laboratories.

Treatment

Treatment is supportive for most respiratory viruses – symptomatic relief, with oxygenation and ventilation if necessary. Specific treatment for influenza and CMV is available.

Prevention

For most respiratory pathogens, hygiene is important, i.e. in the prevention of aerosolised virus and contaminated surfaces. Live attenuated measles and varicella vaccines are available and for influenza both subunit (killed) and live attenuated vaccines are in use. Palivizumab is used to prevent RSV infection.

Key points

- Respiratory syncytial virus (RSV) infection can be prevented in high-risk infants using palivizumab.
- Fomites and aerosols should be prevented in order to limit spread of respiratory viruses.
- Influenza vaccination is a well-established method of protecting people against the annual influenza epidemic.

Hepatitis viruses

Introduction

Viral hepatitis and alcoholic hepatitis are the most common causes of hepatitis (Table 1). Hepatitis B virus (HBV) is one of the most important vaccine preventable diseases worldwide and effective vaccination to HBV has the potential to result in a significant reduction in hepatocellular carcinoma. Hepatitis A virus (HAV) prevention is important for travellers. An overview of the virological, epidemiological and clinical characteristics of the major hepatotropic viruses can be found in Table 2. (HAV, HBV and hepatitis C virus (HCV) virology is discussed in more detail in the picornavirus, hepadnavirus and flavivirus sections respectively.)

Clinical hallmarks of acute viral hepatitis

Patients with acute hepatitis complain of loss of appetite, tiredness, malaise and, in severe cases, they may also have nausea, vomiting, clinically apparent jaundice, dark-orange-coloured urine and a lighter colour of stool. On examination a patient may also have an enlarged and tender liver. Liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) usually have values of more than 500 IU/l, indicating severe damage to the liver cells. When acute hepatitis is accompanied by extensive liver damage, patients may have liver failure. Clinical indications of liver failure are hepatic encephalopathy (altered consciousness and a course tremor called liver flap) and continuous vomiting or a bleeding tendency due to a decrease in clotting factors, which are bad prognostic signs. A rapidly progressive course of acute hepatitis to liver failure is quite rare and is referred to as fulminating hepatitis. Patients with underlying liver damage, old age or pregnant patients are more likely to develop fulminating hepatitis.

Chronic active hepatitis

Chronic viral hepatitis can be defined as viral hepatitis lasting for more than 6 months. Some patients with chronic hepatitis virus infection do not have liver inflammation. These patients are referred to as asymptomatic carriers. When chronic infection is characterised by inflammation and liver cell damage it is referred to as chronic active hepatitis. Chronic active HBV or HCV infection can result in progressive destruction of the liver during a process called cirrhosis, which is characterised by fibrosis and nodular regeneration. Clinically a patient with cirrhosis can have an irregular liver margin on examination and extended veins on the abdomen or in the oesophagus. This can lead to life-threatening haematemesis (vomiting of blood). Long-standing liver cirrhosis can lead to chronic liver failure, which is associated with clinical findings including gynaecomastia, palmar erythema, spider naevi and, on serum biochemistry, decreased serum albumin and increased globulin.

Patients with HBV infection (especially if they have cirrhosis) and patients with cirrhosis due to hepatitis C virus infection are at increased risk of developing hepatocellular carcinoma.

Recent advances in the treatment of chronic active hepatitis both due to HBV and HCV virus has the possibility to prevent liver cirrhosis, chronic liver failure and hepatocellular carcinoma. Nevertheless, primary prevention of these infections remains the most important.

Table 1 **Causes of hepatitis**

Toxins	Alcohol, CCl_4, others
Medication	Anti-tuberculosis, antibiotics, many other
Hepatotropic viruses	Hepatitis A, B, C, D virus
Systemic viral infection	Herpesviruses (HSV, CMV, EBV), adenoviruses, arboviruses (yellow fever, Crimean–Congo haemorrhagic fever, Rift Valley fever)
Severe sepsis	Gram negative bacteria
Ischaemic hepatitis	Shock, including hypovolaemic shock due to trauma
Auto immune hepatitis	Type 1 and Type 2

Treatment of viral hepatitis

Acute viral hepatitis

Treatment is symptomatic and supportive.

Chronic viral hepatitis

Chronic hepatitis B virus infection

The aim of treating chronic HBV infection is primarily to prevent complications since cure is often not achievable.

Evaluation to decide if therapy is indicated includes HBeAg and anti-HBe serology, HBV load, transaminases and liver biopsy. Patients with evidence of significant viraemia and chronic inflammation without cirrhosis will benefit the most from therapy. Since HBV has a reverse transcriptase enzyme it is susceptible to a range of nucleoside reverse transcriptase inhibitors: lamivudine, emtricitabin, telbivudine, tenofovir entecavir and adefovir dipivoxil show response rates of between 20% and 40%. These drugs are well-tolerated, but resistance develops, especially to lamivudine and emtricitabine.

The other alternative for chronic HBV therapy is pegylated interferon therapy. Interferon has the advantage of a higher rate of permanent response but has more side effects and adverse effects than the nucleoside analogues (see section on antivirals).

Chronic hepatitis C virus infection

Patients with chronic HCV infection and elevated liver enzymes and proof of inflammation and fibrosis on biopsy will need therapy. Genotyping is important to establish the prognosis and duration of therapy. Genotypes 1 and 4 respond less favourably. Improved response has been achieved by combining of pegylated interferon-α-2a and ribavirin and recently by combination with HCV protease inhibitors: boceprevir and telaprevir. Unlike with hepatitis B infection, sustained response can be equated to cure.

Which viruses are transmitted enterally and which parenterally? Prevention is better than cure:

- What are the most effective strategies to prevent HBV, HCV and hepatitis D virus infections?
- Who requires HAV vaccination?
- Which patients with viral hepatitis need and would benefit from disease-specific therapy?

Table 2 **Virological and clinical characteristics of the major hepatotropic viruses**

	Hepatitis A virus	Hepatitis B virus	Hepatitis C virus	Hepatitis D virus	Hepatitis E virus
Taxonomy and properties	Family: Picornaviridae Genus: Hepatovirus Size: 27 nm Naked capsid; +ssRNA, 7.5 kb	Family: Hepadnaviridae Genus: Orthohepadnavirus Size: 42–47 nm Enveloped; dsDNA 3.2 kbp	Family: Flaviviridae Genus: Hepacivirus, 6 major genotypes Size: 27 nm Enveloped; +ssRNA, 9.4 kb	Defective RNA virus, Delta virus Size: 36 nm Enveloped Circular –ssRNA; 1.7 kb;	Genus Hepevirus, not assigned to a family Size: 27–34 nm Naked capsid +ssRNA, 7.5 kb
Route of infection	Faeco-oral Family spread Environmental spread	Vertical: MTCT Horizontal among children Sexual Blood and blood products Scarification IV drug use	Vertical: MTCT IV drug use Blood and blood products Sexual	Blood and blood products	Faeco-oral Consumption of pig meat Environmental spread Low secondary attack rate
Infection risk factors	Low socio- economic status (early in life) Day-care centres Sewerage works Shellfish consumption Travel to endemic areas	Living in endemic countries IV drug use Multiple sexual partners	IV drug use Haemophilia Haemodialysis and haemodialysis units Multiple sexual partners	IV drug use Sexual transmission Possible in areas of high endemicity	Drinking contaminated water Eating contaminated shellfish or pig meat Travel to or living in endemic areas
Global prevalence	Worldwide; seroprevalence is low in developed countries	High: Africa, Amazon Basin, Middle East, E. Europe, SE Asia, Pacific region	High: Egypt Intermediate: Saudi Arabia, Ethiopia, Eastern Europe, Central Asia, Russia, Japan	High: Italy, Southern Europe, Middle East, Parts of Africa, South America, Middle East, Taiwan	High: Indian subcontinent, Central Asia, China, Indonesia, North Africa, Somalia, Sudan, Mexico
Incubation period	2–6 weeks (4 weeks average)	6–24 weeks (12–14 average)	2–26 (6–7) weeks	3–7 weeks	2–8 weeks (5–6 weeks average)
Pathogenesis	Virus poorly cytopathic Cytotoxic T-cells induce liver cell necrosis	Complex interaction of virus and immune system leads to hepatocellular damage.	Complex interaction of virus and immune system leads to hepatocellular damage.	Immune-mediated damage. HBV is an essential cofactor	Virus is directly cytopathic
Diagnosis	Serology: EIA: HAV-IgM- acute infection. HAV-IgG: immunity	Serology: EIA or RIA HBsAg: current infection; anti-HBcIgM: acute infection or reactivation; present in window period after HBs loss before anti-HBs appears. Total anti-HBc: Current or previous infection. HBeAg: high infectivity anti-HBs: immunity	EIA: HCV-antibodies HCV-RNA: early diagnosis of infection, cleared x chronic infection; genotyping: choice of appropriate regimen and therapy duration	Detection of delta antigen and anti-delta antibodies in serum	Serology: HEV-IgM-acute infection
Clinical manifestations	Acute hepatitis; severity age-dependent: children under 2 years usually asymptomatic vs. adults over 40 high mortality	Acute hepatitis Chronic asymptomatic carrier Chronic active hepatitis Definition of chronic infection = duration of more than 6 months	Acute hepatitis rare, usually mild Chronic hepatitis	Acute infection more severe/ fulminant than HBV mono–infection	Acute hepatitis Risk of mortality due to fulminant hepatitis increased in pregnancy (20% in third trimester)
Complications	Liver: fulminating hepatitis (old age, underlying liver disease) No extra-hepatic complications No chronic state	Liver: cirrhosis; hepatocellular carcinoma Important extra-hepatic complications: polyarteritis nodosa; glomerulonephritis Risk of chronicity: neonate (90%); child (70–80%); adult: 5%	Liver cirrhosis 30%; hepatocellular carcinoma Important extra-hepatic complications: glomerulonephritis; cryoglobulinaemia; thyroiditis; porphyria cutanea tarda Risk of chronicity: 50–85%	60–70% chronic cases develop liver cirrhosis No extra-hepatic complications Risk of chronicity: co-infection (5%); superinfection (80%)	No extra-hepatic complications
Prevention	Sanitation, clean water and food, hygiene Active immunisation (inactivated vaccine) Passive immunisation Antibody administration for travellers (if <2 weeks before exposure) Post exposure: active and passive immunisation	Avoid high- risk behavior Screening of blood products Vaccination (recombinant or plasma purified S-protein) Post exposure, neonates and occupational exposure (non-immune): active and passive immunisation (hepatitis B hyperimmunoglobulin)	Avoid high risk behavior Vaccinate carrier against hepatitis A virus to prevent severe hepatitis Screening of blood products No HCV vaccine available	Avoid high risk behavior No vaccine available Prevent hepatitis B virus infection	Sanitation, clean water and food No vaccine available

MTCT, mother-to-child transmission

Key points

- Hepatitis B virus and hepatitis A virus infection can be prevented by vaccination.
- Chronic HBV and HCV infection is defined as infection lasting for more than 6 months and can, over time, result in liver cirrhosis, liver failure, hepatocellular carcinoma and extra hepatic complications. Specific therapy for patients with chronic active infection can prevent these complications.

Viral infections of skin and mucosal membranes

Definitions

Exanthem – a rash, usually widespread, on the skin.

Enanthem – a rash on the mucosal membranes.

Measles

The incubation period is 10–11 days and the onset of disease is characterised by a prodrome. Koplik spots are punctate blue-white lesions surrounded by erythematous rings on the buccal mucosa, opposite of the second molars (Fig. 2). They appear 1–2 days prior to the onset of the exanthema and fade after 2–4 days. The typical maculopapular rash (Fig. 1) caused by measles occurs 14 days after exposure and starts on the forehead and in the areas adjacent to the ears. It spreads and involves the rest of the face, neck, trunk and extremities during the following 3 days. The rash fades within 3–4 days and residual brown pigmentation of the skin and desquamation may persist.

Rubella (German measles)

Rubella has an incubation period of 13–20 days. Lymphadenopathy, involving the suboccipital, posterior auricular and cervical lymph nodes, and a viraemia may be present 1 week prior to the onset of the rash. An enanthem may precede the rash by 3 days. The rash is fine and macular (Fig. 3), starting on the face and spreading towards the trunk and extremities. The pinpoint macular lesions coalesce and may be apparent for up to 3 days. Rubella may also give rise to a purpuric rash. One quarter of patients with rubella do not present with a rash.

Parvovirus B19

The virus is the causative agent of fifth disease or *erythema infectiosum*. The incubation period varies between 6 and 18 days. The exanthema follows a prodromal phase that may include respiratory and gastrointestinal symptoms. The rash itself is divided in three stages. The first phase represents the typical 'slapped cheek' appearance and occurs 2–5 days after the onset of the prodrome (Fig. 4). The rash is characterised by circumoral pallor and raised edges. The second stage occurs 1–4 days later and involves an erythematous maculopapular rash, extending over the trunk and extremities. Stage three involves gradual fading of the rash in a lacy, reticular fashion within a period of 1–3 weeks. Erythema infectiosum can be triggered by exercise, a warm bath, sunlight and emotion. Parvovirus B19 infection may also give rise to other dermatological features. These include a glove and stocking syndrome, a vesicular – pustular rash and *erythema multiforme*.

Human herpes virus 6

Human herpes virus (HHV)-6 is the cause of sixth disease or *exanthem subitum* (roseola infantum) and typically infects babies between the ages of 6 and 9 months. Patients present with a 3–4 day history of fever. A maculopapular rash involving the face and trunk may follow and may be accompanied by diarrhoea and transient leucopoenia (see Fig. 1a and 1b in Human herpesviruses 6, 7 and 8 chapter).

Enteroviruses

Enteroviruses are transmitted via the faecal-oral route. The incubation period is 2–40 days. *Herpangina* affects children aged 2–10 years and is characterised by vesicles and/or ulcers on the soft palate. It is caused by coxsackie virus A9 and may result in nausea, vomiting, pharyngitis and odinophagia. Coxsackie A16 and enterovirus 71 can give rise to hand, foot and mouth disease, an exanthema of painful vesicles involving the hands, feet, buttocks and genitals. The disease is also accompanied by fever and a buccal enanthema. Enteroviruses can also present with a fine, rubella-like maculopapular rash (Fig. 5), usually associated with other systemic symptoms.

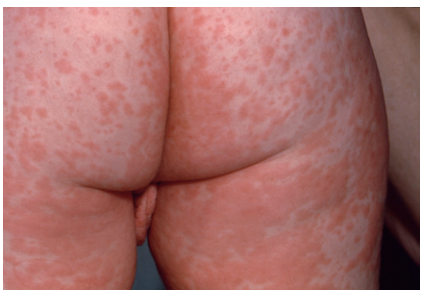


Fig. 1 **Measles.** (Photo courtesy of CDC.)

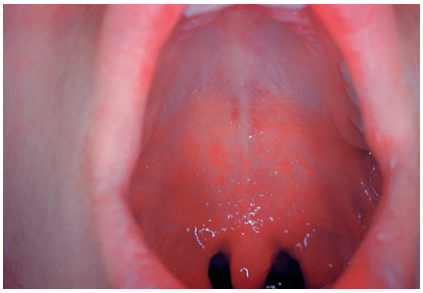


Fig. 2 **Koplik spots.** (Photo courtesy of CDC/ Heinz Eichenwald.)

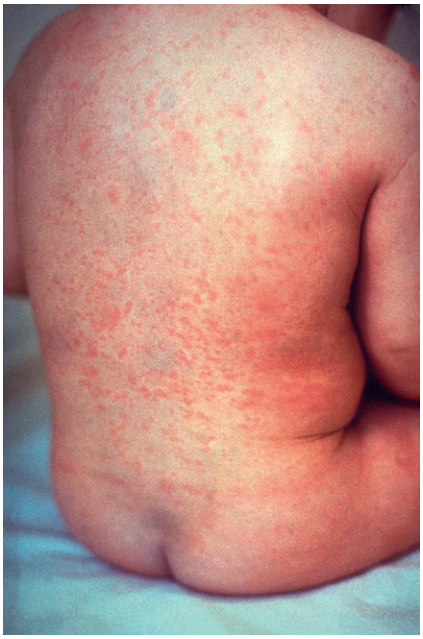


Fig. 3 **Rubella.** (Photo courtesy of CDC.)

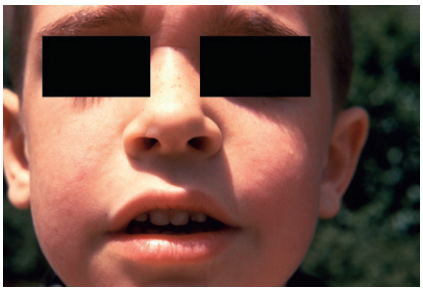


Fig. 4 **Parvovirus B19 infection.** (Photo courtesy of CDC.)

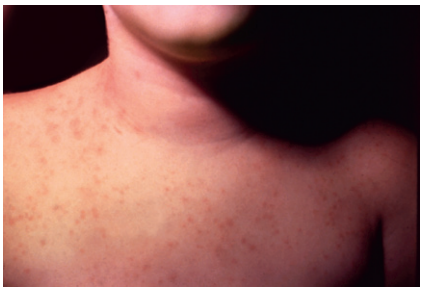


Fig. 5 **Echovirus infection.** (Photo courtesy of CDC.)

Herpes simplex virus 1 and 2

The skin and mucosal involvement of herpes simplex virus (HSV) infection may take on many forms. Primary infection, mostly due to HSV-1, results in gingivostomatitis, characterised by ulcers and vesicles involving the hard palate in particular (in contrast to herpangina). Skin surrounding the mouth is also affected and submandibular lymphadenopathy is present. The lesions eventually become painless, but the condition may last up to 3 weeks. Reactivation takes the form of *herpes labialis* (fever blisters). Herpes labialis (Fig. 8) may be preceded by a prodromal itching and tingling sensation.

Primary genital infection (usually due to HSV-2) may present with fever, urethritis, cystitis, inguinal lymphadenopathy and painful vesicles that can involve the vulva, vagina, cervix, penis or anus. An estimated 60% of primary infections result in recurrences.

HSV dermatitis may present in a number of ways. *Eczema herpeticum* is the result of HSV infection in people with an underlying skin condition (e.g. atopic eczema, Darrier disease, burns). *Herpetic whitlow* follows inoculation of virus in the finger after nail biting or procedures carried out by dentists or anaesthetists. Wrestlers and rugby players may suffer from *herpes gladiatorum* (scrum pox). HSV may also present like zoster, without the accompanying neuralgia (zosteriform herpes simplex). HSV is also a cause of *erythema multiforme*, involving the acral area and taking the form of red papules, concentric target lesions, annular plaques and/or bullae.

Immunosuppressed patients may suffer from disseminated skin infection as well as progressive genital infection.

Varicella zoster virus

Primary infection with varicella results in chickenpox (Fig. 6). The incubation period is 2–23 days. Patients with chickenpox are infective for a period reaching from 2 days before until 5–7 days after the appearance of the rash. By this time, usually, all old vesicles are crusted and no new lesions have appeared. Haemorrhagic chickenpox may complicate the primary infection in immunosuppressed patients.

Herpes zoster is the reactivated form of varicella zoster virus (VZV) infection from latency. The rash (Fig. 7) involves a dermatome(s), corresponding to the affected sensory nerve(s). Zoster occurs in old age, immunosuppressed patients and in infants/children whose mothers developed chickenpox whilst pregnant. Zoster may be preceded by a burning or shooting sensation and even by muscle weakness. Zoster is a common mask for underlying HIV infection and initially only involves one dermatome. It can however involve multiple dermatomes, disseminate and even present as atypical wart-like lesions in patients with stage 4 disease/AIDS.

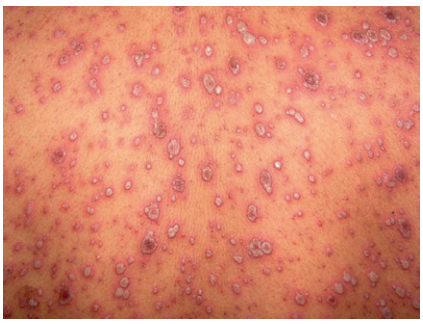


Fig. 6 **Chickenpox.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

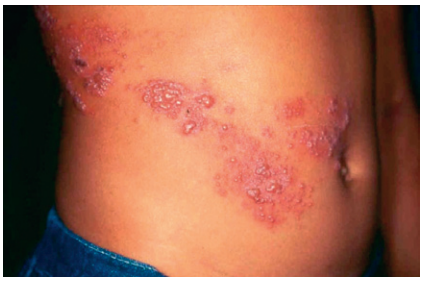


Fig. 7 **Herpes zoster.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

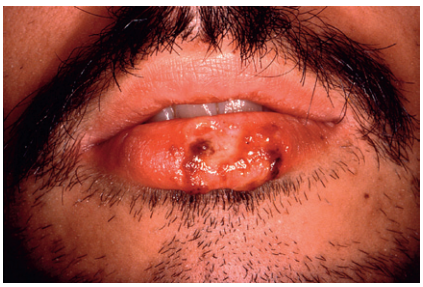


Fig. 8 **Chronic mucocutaneous herpes lesion in a patient with HIV/AIDS.** (Photo courtesy of CDC/Sol Silverman.)

Poxviruses

Smallpox presented with high fever, petechiae, headache, backache and vomiting after an incubation period of 10–14 days. A characteristic rash followed a day or two after the appearance of the prodromal symptoms. Monkeypox, cowpox, tanapox, orf and milker's nodules are the result of infections by poxviruses.

Molluscum contagiosum is a common skin infection caused by the genus *Molluscipox virus*. It may present as papules or discreet, smooth, flesh-colored, dome-shaped nodules with characteristic umbilicated centres. Lesions may be numerous and confluent. The trunk and proximal extremities in children are involved, whereas the pubic areas, thighs and trunks of adults are generally affected. Infection can be severe in immunocompromised patients. (See Fig. 3 in Sexually transmitted viruses chapter.)

Human papilloma virus

Human papilloma virus (HPV) causes warts. It infects the differentiated squamous cell epithelium of keratinised skin and mucosal membranes. HPV-1, 2, 3 and 4 are associated with common exophytic warts, *verucca vulgaris* (see Fig. 2 in Human papillomavirus chapter), involving the hands, fingers, elbows and knees, as well as with endophytic plantar warts. HPV-6 and HPV-11 give rise to *condyloma accuminata* (see Fig. 3 in Human papilloma virus chapter) and may result in obstructive labour in affected individuals and laryngeal papillomas of their newborn babies.

Epidermodisplasia verruciformis (EV) is an autosomal recessive condition, caused by HPV-5 and HPV-8, that presents as multiple, dry, red, confluent flat warts with scaling on sun-exposed parts of the body (e.g. head, neck and hands). Up to one-third of cases develop squamous cell carcinoma of the affected area(s).

HPV is associated with premalignant and malignant genital lesions. High-risk serotypes, HPV-16 and HPV-18, cause cervical carcinoma. HPV is also associated with the development of vulval, vaginal and penile carcinomas. (See Chapter 25.)

Other viruses

A vast array of viruses presents with maculopapular or haemorrhagic rashes, affecting skin and mucosal membranes e.g. arboviruses, haemorrhagic fever viruses and HIV.

Table 1 **Rashes: varicella zoster virus (VZV) vs. smallpox**

VZV	Smallpox (Variola)
Centripetal	Centrifugal
No involvement of palms and soles	Involves palms and soles
No/little systemic (prodromal) symptoms	Systemic (prodromal) symptoms
Superficial, irregular lesions	Deep, round lesions
Different stages of lesions present at any certain time	Only one stage of lesions present at any certain time

Viral infections and pregnancy

Introduction

Two new concepts arise in pregnancy – an altered immune state of the mother, and a fetus that can be infected, either prior to delivery or perinatally. Both of these have significant implications for viral infections, which can cause serious pathology in these cases.

Infections of the fetus

Viruses that cause significant infections of the fetus include cytomegalovirus (CMV), rubella, varicella zoster (VZV) and parvovirus B19. These are acquired by vertical transmission across the placenta, and possibly ascending infection from the genital tract in the case of CMV.

Cytomegalovirus

CMV is a common disease, and between 20 and 100% of women are infected by adulthood, depending on the socioeconomic circumstances. In women with poor socioeconomic circumstances, congenital CMV is a greater problem, as they are more likely to be infected already (CMV remains latent with periodic reactivation). Primary CMV infection during pregnancy has the worst outcome for the fetus, although reactivation or reinfection also has risks (Table 1).

Antenatal diagnosis is possible – detection of IgM or virus in cord blood or amniotic fluid is indicative of infection; ultrasound can indicate congenital defects. Postnatal diagnosis should ideally be made as soon as possible after birth, to exclude later infection, and the generally accepted time is within the first 4 weeks. IgM positivity and CMV excreted in the urine or respiratory tract at birth or soon thereafter indicate congenital infection. Clinical signs and symptoms are listed in Table 2.

Management includes termination of pregnancy for those obviously congenitally abnormal, but is generally not recommended. Gancyclovir during pregnancy is being investigated.

Rubella

Congenital rubella infection is a devastating condition, but the incidence has decreased significantly since the introduction of the rubella vaccine. All women should be vaccinated prior to puberty and the ideal would be to include all males as well, in order to limit circulation and prevent exposure of women to clinical cases.

Rubella virus does not kill cells, but limits their growth and division, thereby interfering with organogenesis.

Clinical appearance

Several transient signs and symptoms are present in the first few weeks of life. These include a cloudy cornea, low birth weight, thrombocytopenia and hepatosplenomegally. Hepatitis and pneumonitis are rare.

Permanent defects occur, some only becoming apparent as the child develops. Developmental concerns are mental retardation, psychomotor retardation and language deficits. Sensory organs are affected – deafness due to damage to the organ of Corti, unilateral or bilateral cataract formation, retinopathy with a salt-and-pepper appearance, glaucoma, myopia and microphthalmia. A progressive subacute panencephalitis has been seen. Endocrine disturbances are seen – usually diabetes mellitus, but growth hormone deficiency and thyroid hormone imbalances can occur. Cardiac defects such as pulmonary valve and peripheral pulmonary vessel stenosis, patent ductus arteriosus and ventricular septum defects. Skin lesions (Fig. 1), inguinal hernias and undescended testes are common.

Diagnosis

Serology: testing for immunity – IgG
Current/recent infection – IgM

If IgM is positive and IgG is positive, an avidity test should be done to determine if the infection is a primary infection or a re-infection – important in diagnosing rubella infection in pregnancy, if no previous sample is available for comparison. Timing of serology is also important – see Table 3.

Isolation: rubella can be cultured on cells, using respiratory samples and urine, as well as amniotic fluid.

In congenital infections, isolation should be done as early as possible after birth, to avoid detecting an early neonatal infection.

Molecular: polymerase chain reaction (PCR) is available for certain indications and is ideal for amniotic fluid.

Table 1 **Cytomegalovirus: Infection risks for fetus**

	Primary infection	Reactivation / reinfection
Risk of infection	30–50%	1%
Infected –> symptomatic at birth	7–10%	1%
Later sequelae	90%	80%
Infected –> asymptomatic at birth	90–93%	99%
Later sequelae	10–15%	10–15%

Table 2 **Cytomegalovirus: Clinical conditions**

Symptoms at birth		
Central nervous system	***Other***	**Sequelae that develop or are noticed later**
■ Microcephaly	■ Chorioretinitis	■ Sensorineural hearing loss
■ Mental retardation	■ Hepatosplenomegaly	■ Severe motor deficit
■ Spasticity	■ Hepatitis, jaundice	■ Mental retardation
■ Epilepsy	■ Pneumonitis, pneumonia	■ Chorioretinitis
■ Periventricular calcifications	■ Congenital heart defects	
■ Sensorineural hearing loss	■ Myocarditis	
■ Encephalitis	■ Thrombocytopenia	
	■ Petechiae	
	■ Inguinal hernia in males	
	■ Anophthalmia	
	■ Prematurity	
	■ Intrauterine growth retardation	
	■ Neonatal death (20% of those symptomatic at birth)	

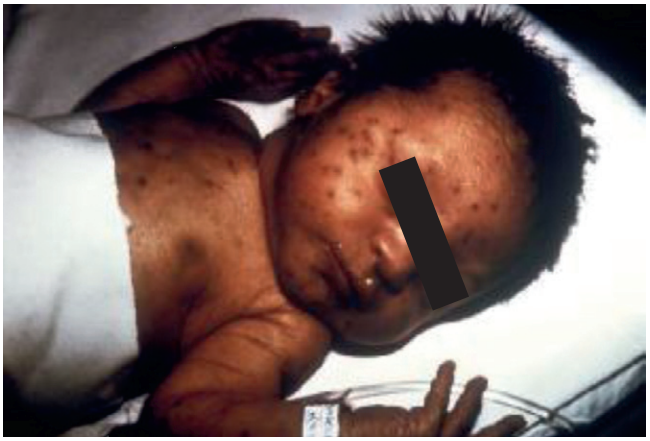


Fig. 1 **Congenital rubella infection showing 'blueberry muffin' skin lesions.** (Photo courtesy of CDC.)

Table 3 **After rubella exposure in a pregnant woman**

<10 days ago	→	IgG +	=	Immune
	→	IgG – IgM –	=	Continue to test weekly
10-21 days ago	→	IgM +	=	Infected
>21 days ago	→	IgM +	=	Infected
	→	IgG – IgM –	=	Uninfected, vaccinate post-delivery

Varicella zoster

Infection in the first trimester results in 1% of infants being infected with varicella, resulting in scarring of the skin, limb hypoplasia, muscular atrophy, rudimentary digits, cortical atrophy, psychomotor retardation, congenital cataracts, chorioretinitis, anophthalmia and gastrointestinal abnormalities. In the second trimester, the infection rate rises to 2%.

Parvovirus B19

The main target cells of parvovirus B19 are erythroid precursor cells, but it also infects endothelial cells and fetal myocardial cells, which explain transplacental transmission, and fetal cardiac involvement.

Fetal infection results in anaemia and myocarditis resulting in cardiac failure and severe oedema, known as hydrops fetalis. In the second trimester, fetal loss is of concern. Congenital malformations are not believed to occur.

Intrauterine blood transfusions may assist the fetus.

Infections of the newborn

Significant viral infections acquired in the perinatal period include varicella zoster, herpes simplex, HIV, hepatitis B and human papillomavirus. HIV is acquired prenatally across the placenta, or along with hepatitis B perinatally via blood and vaginal secretions to which the infant is exposed during labour. HIV is also spread by breastfeeding. Herpes simplex and human papillomavirus are contracted by the infant through exposure to the genital tract. Varicella is acquired in utero. This section needs to be read in conjunction with the chapter on post-exposure prophylaxis.

HIV

HIV in infants can be a rapidly progressive disease – especially for infants infected in utero. 20–30% of infants die within 6 months of birth, while the remainder tend to follow a disease progression much like that of adults and become symptomatic within a few years.

Without antiretroviral prophylaxis, HIV infects 15–35% of newborns, depending on maternal factors such as $CD4^+$ count, viral load, concurrent sexually transmitted infections and type of infant feeding. With antiretroviral treatment for both mother and infant, this can be reduced to 1–2%.

Hepatitis B

The risk of transmission to the infant is highest if the mother has acute HBV infection between the 2nd trimester and 2 months after delivery. In areas where chronic hepatitis B is common, this is also a significant risk for transmission. If the mother is HBV surface antigen positive as well as DNA positive, the risk of the infant being infected is 90%. Neonates usually do not develop symptoms of acute hepatitis, but 80–90% of infants infected perinatally develop chronic infection.

Herpes simplex

Herpes simplex infection of the neonate often presents as disseminated herpes, with liver or cerebral infection. If the mother is known to have genital herpes, a caesarean section should be done.

Varicella zoster

The severity of neonatal VZV depends on the degree to which maternal immunity is transferred to the fetus. If the mother develops VZV seven or more days before delivery, sufficient immunity should be transferred to the infant to prevent serious illness. However, development of a rash after that time leaves the infant unprotected and can result in severe disease. Infants born before 32 weeks' gestation may not have received maternal antibodies and are also at risk. Severe disseminated VZV can occur with untreated mortality rates of about 30%.

Human papillomavirus

Juvenile-onset recurrent respiratory papillomatosis can occur when an infant is born vaginally to a woman with genital papillomata, when the virus is transmitted to the respiratory tract. HPV 6 and 11 are the usual causes, but HPV 16 and 18 have been seen to cause this condition. Prevention takes the form of a caesarean section, while treatment is often surgical, with limited success of cidofovir and ribavirin.

Infections of the pregnant woman

Several viral infections can present with more serious clinical disease in pregnant women. VZV can become disseminated, causing pneumonia, hepatitis or encephalitis, or very severe classical VZV. Hepatitis A and E viruses may present with fulminant, often fatal, hepatitis. Respiratory infections are often worse during pregnancy – pregnancy during the influenza season is an indication for vaccination.

Viruses and cancer

Normal cell growth, differentiation and function are dependent on regulation by complex systems controlling important cellular processes like replication, repair and apoptosis. Cell growth must be regulated in such a way that organs and tissues conform to a certain shape and size and do not undergo continuous hypertrophy when confronted with damage by disease or trauma processes. Mechanisms are therefore needed both for stimulating cellular development when necessary and for suppressing it to prevent uncontrolled overgrowth. The normal cell cycle has 'built in' regulatory controls for different steps and phases.

Oncogenesis can be defined as the progression of cytological, genetic and cellular changes, resulting in the development of a malignant tumour (Fig. 1). Transformation refers to the process that normal cells undergo to become malignant entities and include immortalisation of cells with a loss of contact inhibition between them, as well as decreased dependence of cells on anchorage and exogenous growth factors for their continuous growth and development. Various carcinogens like chemicals, radiation, nicotine, mycotoxins, hormones and viruses may be responsible for altering normal cellular genes and causing mutations that may ultimately lead to transformation. They may act separately or work together in transforming cells. Mutations may result in a *gain of function* (overexpression or stimulation of a certain gene or oncogene) or a *loss of function* of other genes (typically tumour suppressor genes like p53 and pRb, with major functions like inducing programmed cell death or apoptosis and repairing damaged DNA). It is important to remember that cancer is regarded as a multistep process and that more than one mutation acquired over a period of time results in disease. The immune system also plays a vital role in tumour surveillance and cellular immunodeficiency typically contributes indirectly to the oncogenic process.

An estimated 15% of all human cancers are said to have a viral aetiology. This is particularly true in underdeveloped countries where many viral infections are acquired very early in life. Oncogenic viruses (oncoviruses) have two main characteristics that make them ideal for causing cancers in humans. Firstly, oncoviruses typically cause chronic, permanent infections in human cells. Most of these viruses integrate part of their genome randomly into the host cell's genome and may take control of host genes and regulatory elements in this way. Some oncoviruses secondly, are not cytocidal and do not ultimately result in death of the host cell. These viruses are therefore able to transmit possible genetic alterations obtained to their progeny or daughter viruses. Having said this, cancers caused by viruses are mostly not intended and merely a 'side effect' of their normal replicative strategies.

Viruses may cause cancer by utilising direct and indirect mechanisms. Viruses may directly activate cellular oncogenes (*c-onc*) when a part of their genome (provirus) is integrated upstream from the oncogene. Double-stranded DNA viruses and retroviruses may both integrate into the host cell genome. The insertion may be mutagenic, activating the *c-onc* and causing a change in the quality or quantity of subsequent gene expression (insertional mutagenesis). Some viruses capture (transduce) *c-onc* when integrated in the host genome and turn them into true viral oncogenes (*v-onc*). This is the case with Rous sarcoma virus. The virus was discovered in 1911 when a viral oncogen (*src*) was found to transform chicken fibroblasts. Viral gene products may also transactivate *c-onc*s and cause up and down-regulation of various cellular genes. Many oncoviruses exert an effect by mutating and knocking out tumour suppressor genes (p53 and pRb). This causes uncontrolled proliferation of damaged cells due to no repair or apoptotic processes taking place.

Viruses may indirectly be factors in the oncogenesis process. Viruses, like HIV, cause marked depression of cellular immune mechanisms and may, together with viruses like HTLV-1, infect immune cells (CD 4 lymphocytes) directly. Immunosuppression may also hinder the early detection of certain cancers. An attributing factor in the development of hepatocellular carcinoma after infection with hepatitis B or C virus may be the widespread cell regeneration and proliferation taking place after liver cirrhosis has occurred.

Oncoviruses include both DNA and RNA viruses. They will now be discussed separately.

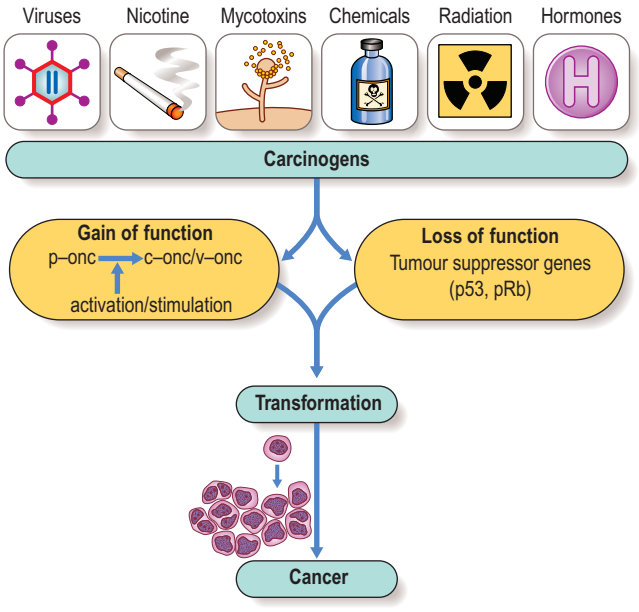


Fig. 1 **Outline of the mechanism of oncogenesis.**

DNA viruses

Papilloma viruses: human papillomavirus

Cervical cancer, various anogenital cancers, laryngeal carcinoma, skin cancer and certain head and neck tumours are associated with human papillomavirus (HPV). 'High'-risk genotypes include HPV 16, 18, 31 and 45. HPV causes cancer by knocking out tumour suppressor genes (p53 and pRb) via binding of E6 and E7 viral proteins to them. The expression of E6 and E7 are up-regulated, once viral integration has occurred. HPV also evades the surveying immune system by expressing late, structural proteins only in proliferating epithelial layers above the basal membrane – a site that is generally immune-privileged and protected from the surveying immune system.

Herpes viruses: Epstein-Barr virus

Epstein-Barr virus (EBV) is a gammaherpesvirus and causes widespread immortalisation of B cells and a polyclonal antibody response. Cytotoxic T cells usually control the B cell proliferation when the immune system is intact. Cancers due to EBV infection are linked to the absence of a sufficient cell-mediated immune response (CMI). Burkitt lymphoma (BL) occurs in children from tropical Africa and Papua New Guinea, and typically presents as a tumour affecting the jaw line. It occurs in areas where the CMI of the population is depressed, due to the burden of *Plasmodium falciparum* malaria. BL is the result of a chromosomal translocation t (8:14/22/2) between the *c-onc* (*c-myc*) on chromosome 8 and the immunoglobulin heavy and light chains on chromosomes 14 (heavy) or 2 and 22 (light). Because of ongoing B cell proliferation and antibody production, the *c-myc* is placed under control of the immunoglobulin genes, resulting in over expression of the oncogen. EBV also deregulates tumour suppressor genes by using a nuclear protein, EBNA 5. Transactivation of cellular oncogenes have been described. Another EBV protein, LMP 1, activates bcl-2, resulting in decreased apoptosis. Other cancers caused by EBV include: nasopharyngeal carcinoma, Hodgkin disease, non-Hodgkin lymphoma and primary central nervous system lymphoma in HIV/AIDS patients. EBV is also the cause of post transplant lymphoproliferative disease (PTLD) and X- linked lymphoproliferative disease (Duncan syndrome).

HHV8/ KSHV

HHV 8 was first identified in 1994 in tissues from AIDS patients with Kaposi sarcoma, a tumour affecting endothelial and spindle cells. Various viral products are associated with the development of cancers by inducing cellular oncogenes as well as by altering tumour suppressor genes. These products include LANA (latency product), a bcl 2 homologue, an Il-6 homologue, and a viral cyclin. HHV 8 is also associated with primary effusion or body cavity lymphoma and multicentric Castleman's disease.

Hepadnavirus: hepatitis B

Hepatitis B is a cause of hepatocellular carcinoma (HCC) and factors like the presence of chronic cirrhosis and acquisition of the infection in early childhood predispose to the development of cancer. Hepatitis B may cause HCC by employing different strategies. Random insertional mutagenesis, *c-myc* deregulation, inactivation of tumour suppressor genes as well as transactivation of cellular oncogenes by hepatitis B X protein are direct ways by which the virus operates in transforming cells. The indirect way via cell regeneration has already been mentioned. HCC due to hepatitis B can largely be prevented by active immunisation.

RNA viruses

Retroviruses: HTLV-1

HTLV-1 is the causative agent implicated in ATLL (adult T-cell leukaemia/lymphoma). ATLL primarily affects people of Japanese origin and typically presents many years after the initial infection has occurred. HTLV-1 employs a transactivation strategy via two viral proteins, termed *Tax* and *Rex*. *Tax* upregulates the expression of IL 2, a potent T cell proliferation activator. This results in an uncontrolled population of clonally expanded T cells.

HIV

HIV is not regarded as a true oncovirus, but may be a cofactor in oncogenic processes by depressing cell-mediated immunity and allowing opportunistic cancers to develop.

Flaviviruses: hepatitis C

Hepatitis C represents another cause of HCC. The mechanism of oncogenesis may be linked to a non-structural viral gene. Hepatitis C may also cause certain B cell lymphomas.

Other viruses

Adenoviruses and polyomaviruses are implicated in the development of cancers in various animals. To date no human cancers have been attributed to them, although a causative link may exist between JC virus and human colorectal carcinoma.

Key points

- *Oncogenesis* can be defined as the progression of cytological, genetic and cellular changes, resulting in the development of a malignant tumour.
- *Transformation* refers to the process that normal cells undergo to become malignant entities.
- Mutations may cause a 'gain in function' of certain oncogenes or a 'loss of function' of tumour suppressor genes like p53 and pRb.
- Cancer is generally regarded as a multistep process and more than one mutation acquired over a period of time results in disease.
- The immune system plays a vital role in tumour surveillance and cellular immunodeficiency may contribute indirectly to the oncogenic process.
- An estimated 15% of all human cancers are believed to have a viral aetiology.
- Viruses may cause cancer by utilising direct and indirect mechanisms.
- Direct strategies used by viruses to transform cells include insertional mutagenesis, transduction of *v-onc*s and transactivation of various viral genes.
- Human oncoviruses include HPV, EBV, HHV 8, HTLV, hepatitis B and hepatitis C.

Human immunodeficiency virus

Over the past 3 decades the world has witnessed the evolution of the HIV pandemic. The impact of this infection continues to devastate much of Africa and many other poor communities throughout the world. The immunosuppression and immune dysregulation that typifies this disease is triggered by the human immunodeficiency virus (HIV), of which there are two subtypes: HIV-1 and HIV-2. HIV-1 has been responsible for the majority of infections worldwide, whilst HIV-2 causes a milder disease and has affected predominantly those in West Africa.

Virus

HIV belongs to the family *Retroviridae*, so called because of its possession of a reverse transcriptase enzyme, which enables it to produce DNA from genomic RNA. Both HIV-1 and HIV-2 belong to the genus *Lentivirus*. *Lentus* means 'slow' in Latin referring to the insidious onset of clinical signs. HIV-1 is made up of four groups, the most common being group M or 'main' group (Fig. 3).

HIV is 120 nm in diameter with a lipid envelope penetrated by 72 spikes or glycoproteins (Fig. 1). These complex structures interact with receptors on the host cell to facilitate HIV entry. HIV predominantly infects CD4 lymphocytes and gains entry to these cells via two viral envelope proteins gp120 and gp41, aided by a co-receptor CCR5 or CXCR4 receptor. It is thought that as disease progresses, there is a switch from CCR5 to CXCR4 tropic viruses. HIV can also enter cells by endocytosis. Fig. 2 shows the life cycle of HIV. The average viral life cycle takes 1–2 days, with up to 10 billion virions being produced each day. In an untreated person between 10^3 and 10^6 virions are circulating in the plasma at any one time, with concentrations in the lymph nodes being two to three orders of magnitude greater. HIV has a diploid positive sense single-stranded RNA genome, which has nine genes encoding a number of different proteins. The major structural genes are *gag, pol* and *env*. The major regulatory genes are *tat* and *rev*. The accessory genes are *nef, vif, vpr* and *vpu* (or *vpx* in the case of HIV-2).

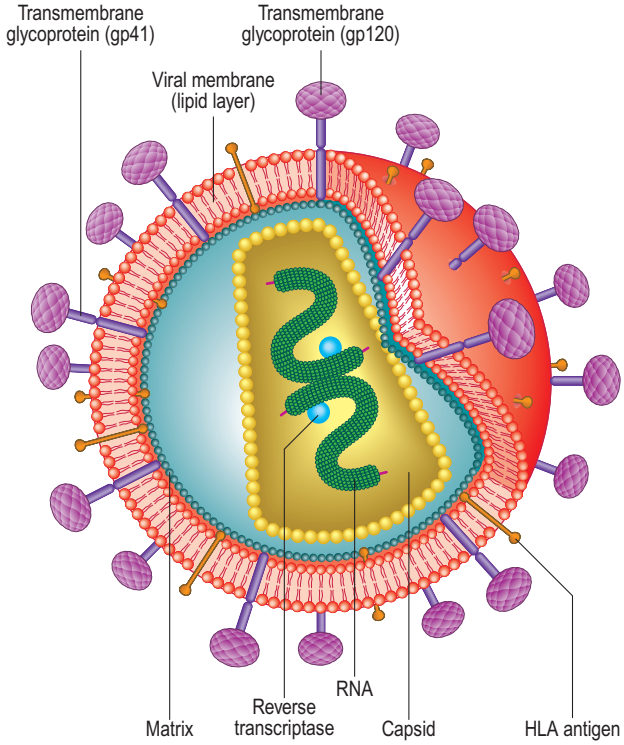


Fig. 1 **HIV structure.**

Epidemiology of HIV

An estimated 34 million people are living with HIV worldwide. This is a 17% increased prevalence from 2001. Although this reflects an unacceptable number of new infections (~2.7 million in 2010), it also reflects increased longevity due to increased access to antiretroviral therapy. The vast majority of infections remain in sub-Saharan Africa where more than 25% of the population may be infected in some areas. Infection rates in many resource rich countries are stable at less than 1%, with infection predominantly localised to high risk groups e.g. men who have sex with men and injecting drug users. Whilst infections in other areas e.g. Eastern Europe and Central Asia have shown dramatic increases in the number of new infections. More than 30 years into the HIV pandemic, HIV remains in a state of flux despite unprecedented investment.

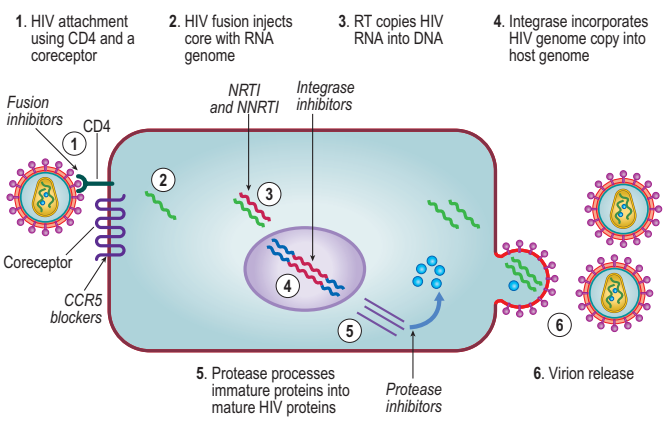


Fig. 2 **Life cycle of HIV, showing antiretroviral drug sites of action.**

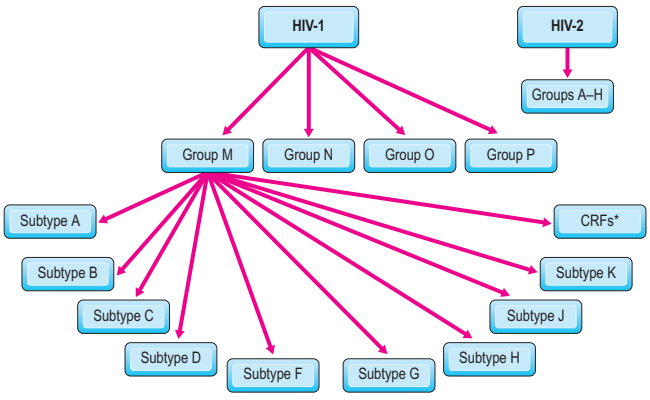


Fig. 3 **Classification of HIV**
*CRF = circulating recombinant forms.

STOP THINK

- Why is it necessary to treat HIV with a multidrug regimens and to ensure high compliance rates?
- What are the ways in which HIV can be prevented?
- At what points in the replications cycle to HIV do the various available drugs act?
- How is HIV diagnosed in an infant?

Clinical

Acute HIV infection is recognised in only about 30% of those who are infected with HIV. It occurs about 6 weeks following exposure and is characterised by lymphadenopathy, rash, malaise and fevers. These symptoms usually settle and, unless HIV testing is initiated, the patients may remain oblivious to infection for many years (Table 1).

Over time the virus compromises the immune system and the patient becomes vulnerable to infections. The type of infection often indicates the degree of immunosuppression. Tuberculosis is common in patients with HIV irrespective of CD4 count, and is an important cause of mortality and morbidity in HIV patients in developing countries. Early on in the course of disease, reactivation of multidermatomal herpes zoster (shingles) or recurrent bacterial infections may occur. Severe immunosuppression (CD4 <200 cells/μl) is associated with cytomegalovirus retinitis, Kaposi's sarcoma (Fig. 4), oesophageal thrush, which causes severe dysphagia (Fig. 5), and pneumonia caused by *Pneumocystis jirovecii*.

Once on HAART, co-morbid illnesses like hepatitis B and C infection and the development of malignancies like cervical carcinoma have an important impact on morbidity and mortality.

Fig. 6 shows the natural history of HIV infection.

Diagnosis

A number of different laboratory techniques are utilised to diagnose and monitor HIV infection (Box 1). Specific antibody to HIV is produced shortly after infection. The earliest time at which antigen may be detected is dependent on a number of variables, e.g. the mode of infection, viral load and immune status of the patient. In most individuals, using the fourth-generation enzyme-linked immunosorbent assay (ELISA) assays, diagnosis may be made within 2–3 weeks of infection. These fourth-generation assays detect both antibody and antigen. In areas of low prevalence for HIV, laboratory-based testing usually makes use of at least two different ELISA assays to confirm a positive test. Immunoblot testing may be used to differentiate HIV-1 from HIV-2 infection. This technique detects antibodies against specific HIV-1 or HIV-2 proteins on a nitrocellulose

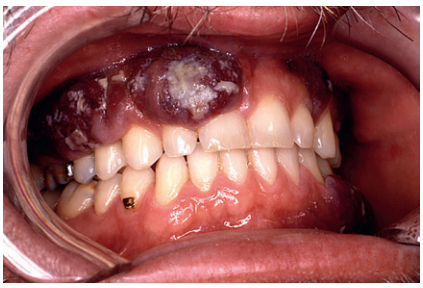


Fig. 4 **Kaposi's sarcoma in a patient with AIDS.** (Photo courtesy of CDC/Dr Sol Silverman.)

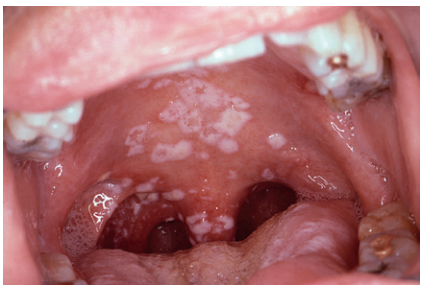


Fig. 5 **Oral thrush in a patient with HIV and a CD4 count of <100 cells/uL.** (Photo courtesy of CDC/Dr Sol Silverman.)

Table 1 **World Health Organization (WHO) clinical staging of HIV/AIDS for adults**

Stage 1	**Stage 4**
Acute retroviral illness	HIV wasting
Asymptomatic	PJP (*Pneumocystis jirovecii* pneumonia)
Stage 2	Recurrent severe bacterial pneumonia
Unintentional weight loss <10% body weight	CNS toxoplasmosis
Angular cheilitis	Cryptosporidiosis diarrhoea >1 month
Recurrent oral ulceration	Isosporiasis diarrhoea
Papular pruritic eruptions	Cytomegalovirus infection (other than liver, spleen, lymph node)
Fungal nail infections	Herpes simplex infection (visceral or >1 month mucocutaneous)
Herpes zoster within last 5 years	Progressive multifocal leucoencephalopathy
Recurrent upper respiratory infection	Disseminated mycosis
Stage 3	Candidiasis of oesophagus, trachea or lungs
Unintentional weight loss >10% body weight	Atypical mycobacteriosis – disseminated
Chronic diarrhoea >1 month	Recurrent non-typhoidal Salmonella septicaemia
Prolonged fever >1 month	Extra-pulmonary tuberculosis
Persistent oral candidiasis	Lymphoma (cerebral or B-cell non-Hodgkin)
Oral hairy leukoplakia	Kaposi's sarcoma
Pulmonary TB (current)	HIV encephalopathy
Severe bacterial infections	Recurrent pneumonia
Unexplained anaemia	Symptomatic HIV-associated nephropathy
Neutropenia	Symptomatic HIV-associated cardiomyopathy
Chronic thrombocytopenia	Reactivation of American trypanosomiasis
	Invasive cervical carcinoma

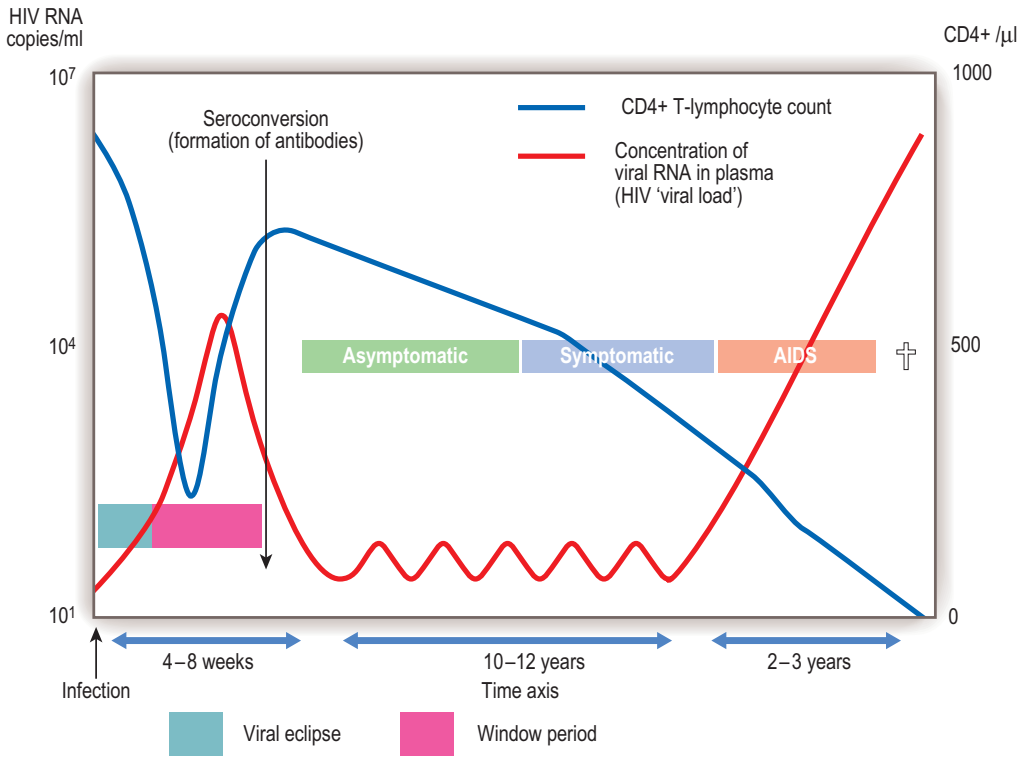


Fig. 6 **The natural history of HIV infection.**

Box 1 **Different HIV testing protocols**

	Scenario	→	Test
1.	Diagnosis of an HIV infection	→	Antibody (+ p24 antigen)
2.	Screening (e.g. VCT, antenatally, blood donors)	→	Antibody (+ antigen/nucleic acid)
3.	Epidemiological surveillance	→	HIV antibody (plus incidence testing)
4.	Special situations (vertical transmission mother to child, occasionally acute primary infection)	→	Qualitative nucleic acid testing (NAT) (usually proviral DNA)
5.	Evaluation of a known infected patient (prognostic marker, monitoring of patients on antiretroviral treatment)	→	Quantitative NAT = 'viral load' (viral RNA copies per volume of plasma); genotyping (detection of drug-resistance-associated mutations)

strip. Rapid tests using immunochromatography are used extensively in resource-poor settings and are increasingly used in the developed world to test hard-to-reach populations, e.g. drug users.

HIV viral load testing is used to monitor therapy. Polymerase chain reaction assay (PCR) and nucleic acid sequence-based amplification assay (NASBA) are the two most commonly used methods to monitor HIV viral load. The aim of therapy is to reduce the viral load in the blood to such low levels that it is not detectable (less than 50 copies/ml). This should be achievable within approximately 16–24 weeks of starting therapy. Patients on therapy are monitored periodically for HIV viral load to ensure that the virus is not replicating in the presence of anti-retroviral drugs.

In infants, since the HIV antibody from their mother is detectable in blood, a molecular test which detects proviral DNA, that is virus which has integrated into the genome, is used. For confirmation HIV viral load may be used in this group, but great care needs to be taken to prevent contamination of samples causing false-positive results. The use of rapid tests in babies and young children has been shown to be of little benefit, as it is associated with an unacceptable level of false-negative and false-positive results.

Treatment

The development of effective therapies for HIV has transformed this disease into a chronic treatable illness. Treatment for HIV so-called highly active retroviral therapy (HAART) usually consists of three different antiretrovirals (Table 2) from at least two different classes. These drugs need to be taken life-long in order to maintain viral suppression. The use of drugs from different classes reduces the risk of the early emergence of resistance. With the discovery of new drugs, antiretrovirals have become easier to tolerate with a lower pill burden, few side effects and even once daily dosing. There continues to be much debate about the benefits of starting therapy early, with some places in the developed world offering therapy irrespective of CD4 count. Sadly for the majority of those affected by HIV, HAART is still restricted to those with CD4 counts of <200 cells/µl.

The development of resistance is a major obstacle to long-term therapy with HAART. Viruses with drug resistance are the result of interplay between HIV viral diversity, HIV replication rate and selective drug pressure. Those who are most at risk are those who are poorly

Table 2 **Antiretroviral drugs**

Nucleoside/nucleotide* reverse transcriptase inhibitors (NRTI/NtRTI*)	Zidovudine (AZT), Stavudine (d4T), Didanosine (ddI), Lamivudine (3TC), Emtracitabine (FTC), Tenofovir* (TDF), Abacavir (ABC)
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Efavirenz, Nevirapine, Etravirine
Protease inhibitors (PI)	Lopinavir, Ritonovir†, Darunavir, Atazanavir, Tipranavir, Fosamprenavir, Indinavir
Integrase inhibitors	Raltegravir
Entry inhibitors	Enfurvitide
CCR5 blockers	Maraviroc

†Ritonovir, although developed as a PI it is now only used in low dose as a pharmacokinetic booster for many of the PIs, allowing lower doses to be used. Specifically it inhibits cytochrome P450-CA4. *Tenofovir is a nucleotide reverse transcriptase inhibitor (NtRTI).

Table 3 **Adverse reactions of antiretroviral drugs**

Short term	**Long term**
Hepatitis	Drug resistance
Stevens Johnson syndrome	Cardiovascular incidents (stroke, myocardial infarct)
Diarrhoea	Diabetes
Lipoatrophy/lipodystrophy	Dyslipidaemia
Lactic acidosis	Osteonecrosis
Renal impairment/failure	

adherent to their medications, whilst transmitted resistance occurs when drug resistant virus infects another, e.g. a baby from a mother with drug resistance. Specific mutations or groups of mutations have been identified and are associated with resistance to specific drugs, e.g. TAMs (thymidine analogue mutations) causing resistance to AZT and other thymidine analogues. Cardiovascular side effects (Table 3) of medication are of increasing concern – with strokes, heart attacks, abnormal lipid profile and diabetes potentially problematic in those who have been on long-term therapy.

Transmission and prevention

HIV may be transmitted from mother to child in utero, prenatally or through breast milk. Without any prophylactic measures approximately 30% of babies born to HIV-infected women will be infected. HIV may be transmitted through sex with someone who is infected with HIV, with insertive anal sex carrying the greatest risk. HIV may be transmitted though needles or other drug using paraphenalia that has been used by someone who is HIV positive. Blood, blood products and unsafe use of needles are all potential iatrogenic routes of HIV infection.

Antiretroviral therapy has been the major weapon in the battle to reduce mother-to-child transmission of HIV. It is also used for the prevention of HIV infection following sexual exposure to the virus in serodiscordant couples or high risk sexual exposure, e.g. rape. It is used to reduce the risk of infection following occupational exposure to HIV, e.g. needlestick injury. Much attention has been given to the modelling data which shows dramatic reductions in the incidence of HIV if all those who are diagnosed with HIV are treated with HAART. The theory behind this is that HAART reduces viral load and, therefore, reduces the risk of HIV transmission. Attention to the safe use of blood products and other potential iatrogenic routes of infection (e.g. re-use of needles) and the availability of needle exchanges to prevent transmission through unsafe injecting, are all important, as are reducing risk through unsafe sex (e.g. reducing partner numbers – particularly concurrent partners and encouraging the use of condoms). In some parts of the world HIV-positive mothers may be advised to have a caesarean section and not to breast feed. In many parts of the world the risks of these practices outweigh the benefits. Circumcision reduces the risk of HIV transmission and encouraging data are emerging on the use of microbiocides, but their usefulness in large-scale public health programmes remains to be seen.

Vaccines

Although there has been some progress in the development of an HIV vaccine, the challenge of HIV vaccine discovery is unprecedented, with the most notable development to date being the Thai RV144 trial, which suggested that protective immunity can be elicited by a vaccine. A number of different factors underlie these difficulties – the integration of HIV proviral DNA into the host-cell genome, the early establishment of latent reservoirs, the enormous genetic and changing antigenic diversity of HIV-1, the failure of inducing broadly reactive antibody responses and the lack of clear immune correlates of protection. The development of a safe, effective and affordable HIV vaccine remains a goal for the foreseeable future.

Key points

- HIV is a retrovirus, which replicates by reverse transcription.
- Diagnosis of HIV in adults is by antibody testing, although most laboratories use a 'dual assay' which detects HIV antigen and antibody.
- HIV can be treated with multidrug regimens which prevent viral replication by acting at various points in the replication cycle.
- Patients started on HAART are monitored using HIV viral load.

Viral haemorrhagic fevers

Viral haemorrhagic fevers (VHFs) are a group of diseases caused by several distinct virus families. The term 'viral haemorrhagic fever' is a term used to denote the clinical presentation of a multisystem syndrome where the vascular system is damaged resulting in severe, uncontrolled haemorrhage. Other characteristics of the syndrome include fever, malaise, vomiting, oedema and hypotension. Although some of these viruses cause mild disease, many cause severe, contagious life-threatening disease. Haemorrhagic fevers are not limited to viruses, other infectious organisms, e.g. scrub typhus, may cause a similar presentation.

Virology

VHF is caused by four distinct viral families: Arenaviridae, Bunyaviridae, Filoviridae, Flaviviridae.

These viruses all share some similar features:

1. Enveloped RNA viruses
2. Zoonoses and have an animal reservoir of infection*
3. Geographically restricted to location of host
4. No cure or proven drug treatment for most of these infections.

The viruses associated with VHF are zoonotic. Their natural host is the animal reservoir or arthropod vector. They are, therefore, completely dependent on their hosts for replication and overall survival. Man is just an incidental host.*

Epidemiology

Historically the distribution of each of these viruses is restricted to the habitat of the particular host. Some hosts live in a limited area, restricting the risk of infection, e.g. with New World arenaviruses, to these areas. Other virus hosts live over much broader areas, e.g. the rodents that carry hantaviruses, may be found all over North and South America. Some hosts like the common rat are distributed worldwide.

However, occasionally infections occur outside of the area where the host lives, often when a host has been exported out of its natural environment. For example Marburg virus which caused disease in laboratory workers who handled infected animals. Infection may also be exported elsewhere. For example an ill traveller may return home and infect a health-care worker. Increasing globalization has resulted in more infections being seen outside of their natural environment, making a thorough travel history a vital part of any medical history taking.

Transmission

Viruses are transmitted to humans when the habitat of humans overlaps with that of the natural host. For example, contact with urine, faeces, saliva or other bodily fluids from an infected rodent. Viruses associated with arthropod vectors are spread when the mosquito or tick bites a human. Some of these vectors transmit to animals which may then transmit to humans when they have contact with infected secondary hosts, e.g. Crimean-Congo haemorrhagic fever (CCHF), Rift Valley Fever (RVF).

Some of these viruses can spread through human-to-human contact. Ebola, Marburg, Lassa and CCHF (Fig. 1) are some examples. This type of transmission can occur directly through close contact with an infected individual – contaminated needles and syringes and body fluids are the most common sources of infection.

Clinical presentation

The specific sign and symptoms vary by disease, but initial signs include fever, fatigue, dizziness, muscle aches, loss of strength and exhaustion. Disease may progress to a haemorrhagic phase when bleeding occurs into the skin, at injection sites, in the internal organs and from body orifices like the mouth, eyes, ears. Severely ill patients show may develop shock, seizures or encephalitis. Some forms of VHF are associated with renal failure (Figure 1).

Treatment

Supportive treatment is standard for most of the VHFs. Ribavirin has been effective in the treatment of some individuals with Lassa or hantavirus pulmonary/renal syndrome. Treatment using convalescent phase plasma has been used with some success in some patients with Argentine haemorrhagic fever.

Control

With the exception of yellow fever, Argentine haemorrhagic fever and Rift Valley fever, for which vaccines have been developed, there are no vaccines to prevent VHF. Therefore, prevention is focussed on avoiding contact with the host species or vector. If a case occurs, the infected individual should be isolated and stringent infection control procedures implemented to prevent onward transmission.

Fig. 1 **A patient with Crimean–Congo haemorrhagic fever virus infection.** (Photo courtesy of CDC/BE Henderson.)

Urban dengue is an exception to these two points.

Table 1 **Classifications**

Family	Disease	Virus	Vector	Distribution
Arenaviridae	Lassa fever	Lassa fever	Rodent	Africa
	Argentine haemorrhagic fever	Junin	Rodent	South America
	Bolivian haemorrhagic fever	Machupo	Rodent	South America
	Brazilian haemorrhagic fever	Sabia	Rodent	South America
	Venezuelan haemorrhagic fever	Guarnarito	Rodent	South America
	Chapare haemorrhagic fever	Chapare	Possibly rodent	South America
	Lujo haemorrhagic fever	Lujo virus	Unknown	Southern Africa
Bunyaviridae	Rift Valley fever	Rift Valley	Mosquito	Africa
	Haemorrhagic fever with renal syndrome	Hantavirus	Rodent	Worldwide
	Crimean–Congo haemorrhagic fever	Crimean–Congo	Tick	Ukraine, Southern Africa, Middle East
Filoviridae	Marburg haemorrhagic fever	Marburg	Possibly bats	Africa
	Ebola haemorrhagic fever	Ebola	Possibly bats	Africa
Flavivirus	Yellow fever	Yellow fever	Mosquito	Tropical Africa, South America
	Dengue haemorrhagic fever	Dengue	Mosquito	Asia, Americas, Africa
	Omsk haemorrhagic fever	Omsk	Tick	Russia
	Kyasanar Forest disease	Kyasanar Forest disease	Tick	South Asia

- Where in the world are you at risk of acquiring viral haemorrhagic fever?

For rodent vectors:

1. Control rodent populations
2. Discourage rodents from nesting or leaving homes or workplaces
3. Encourage safe clean up of rodent nests and droppings.

For arthropod vectors:

1. Insect control
2. Insect repellent
3. Nets
4. Protective clothing>

Infection control (Fig. 2):

1. Avoid contact with body fluids
2. Barrier nurse
3. Disinfect contaminated articles
4. Care taken with equipment which has had contact with infected patients.

Biological weapons

Haemorrhagic fever viruses have been used as biological weapons in the past by world powers and by cult groups. In 1999 CDC classified HFVs as category A bioweapon, based on the potential for person-to-person transmission, their potential to cause widespread illness and death, potential for major public health impact and the necessity for special action for public health preparedness. The threat of the use of these agents heightened after 9/11. Many countries have clear guidelines on reporting procedures should a suspected case of VHF be identified. This is not only to ensure optimum management of the case and to ensure the risks of onward transmission are reduced, but also to ensure that emergency steps can be put in place to control a potential biowarfare attack.

Fig. 2 **Sanitary procedures being practiced in a Kikwit, Zaire clinic during the country's 1995 Ebola outbreak.** (Photo courtesy of CDC/ Ethleen Lloyd.)

Key points

- Viral haemorrhagic fever (VHF) is caused by four distinct viral families: Arenaviridae, Bunyaviridae, Filoviridae, Flaviviridae.
- These viruses are all enveloped RNA viruses, are zoonoses and have an animal reservoir of infection, are geographically restricted to location of host and no cure or proven drug treatment is available for most of these infections.
- Initial presentation may be non-specific, thus a detailed travel history is important.
- Prevention is focussed on prevention of contact with the vector transmitting these infections.

Sexually transmitted viral infections

Introduction

Viral infections of the genital tract account for significant morbidity and mortality worldwide, and affect both partners and children of infected persons, as well as industries such as the sex worker industry, and the trucking industry where sexually transmitted infections are well-studied.

Most infectious agents can spread via sexual contact – if personal contact can result in transmission, closer contact is theoretically more likely to result in transmission. On the other hand, various forms of non-sexual contact can result in transmission of most organisms considered to cause sexually transmissable infections, and may in fact be more common than sexual spread. Sexually transmissable infections are therefore generally considered to be those where the sexual route is the usual route of spread, or where the sexual route of spread is significant enough to warrant intervention.

Specific viral infections

HIV

For details, please see the chapters on HIV.

Epidemiology

HIV is found worldwide and currently about 40 million people are infected, of whom over 60% live in sub-Saharan Africa. Spread is mainly by sexual contact, with mother-to-child transmission also making a significant impact. Other means of spread include shared IV needles for drug use and rare cases of transfusion/transplant associated transmission.

Clinical features

HIV infection has an acute phase, which is symptomatic in about 50% of cases, usually with non-specific symptoms. This is followed by a period of relative health, as the immune system deteriorates over several years, leading to clinical illness in the later stages of disease.

Treatment

Standard treatment is triple therapy with antiretroviral drugs – highly active antiretroviral therapy (HAART). Resistant strains of drugs can be transmitted sexually.

Herpes simplex

Epidemiology

Herpes simplex types 1 and 2 are found worldwide, only in humans. Herpes simplex type 2 has traditionally been associated with genital herpes, and type 1 with oral herpes, but type 1 is believed to cause 20–25% of genital herpes cases. Seroprevalence of HSV-1 worldwide is about 90%, although in developed countries it is below 70%. HSV-2 has a seroprevalence of about 25% in some developed countries, going up to 95% in sex workers. It is believed that HSV-2 seroprevalence is lower than for HSV-1 because of partial protection supplied by an initial HSV-1 infection, and due to sexual activity causing fewer exposures to HSV-2 than kissing causes for HSV-1.

Clinical features

About 80% of those who are seropositive for HSV-2 report remembering a primary infection (Fig. 1) or experiencing recurrent genital herpes. When the clinical disease of primary infection does manifest, it can range from mild to relatively severe, with symptoms of fever, malaise, dysuria and local inguinal lymphadenopathy. Vesicular lesions appear on the base of the glans and shaft of the penis in men, and on the vulva in women, but may be more widespread. The condition may last up to 3 weeks.

Once infected, the virus becomes dormant in the sacral ganglia and may reactivate. Recurrent episodes, reported in about 60% of those who are known to be infected, are less severe and usually associated with a few vesicles causing irritation. Reactivation and viral shedding may occur without symptoms. Complications, which are more common in primary infection, include secondary bacterial infection, and meningitis, encephalitis and sacral radiculomyelitis. HSV-1 infection is partially protective, and if an HSV-2 infection does occur in an HSV-1 infected person, symptoms are usually milder than those of an HSV-1 naïve person.

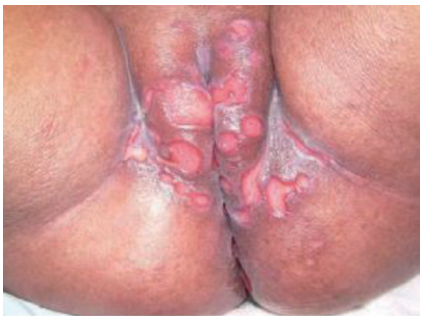


Fig. 1 **Primary HSV-2 infection – herpes labialis.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

Treatment

Acyclovir, valacyclovir, pencyclovir and famcyclovir can be used to treat infection, or to suppress recurrence in patients who experience regular episodes. In cases of resistance, gancyclovir or foscarnet can be used, but resistance in immunocompetent individuals is extremely rare and hardly ever is such a strain transmitted.

Hepatitis B

Epidemiology

Approximately 1 in 20 people worldwide are infected with HBV, most of whom reside in the East. Hepatitis B virus (HBV) is highly infectious. In developing countries, most infection occurs in children, but HBV is relevant to adults worldwide.

Clinical features

Acute hepatitis may occur, followed in some patients by chronic hepatitis B infection. For details, please see the chapter on hepatitis B.

Treatment

For details, please see the chapter on hepatitis B.

Human papillomavirus

Epidemiology

There are more than 100 human papillomavirus (HPV) types known, of which more than 30% involve the genitals. HPV infection of the genital tract is common and varies with age and lifestyle – 5–45% of women have detectable HPV on cervical smears with normal cytology. The common types associated with papillomata without malignant potential are 6 and 11, and those with malignant potential include 16, 18, 31 and 45.

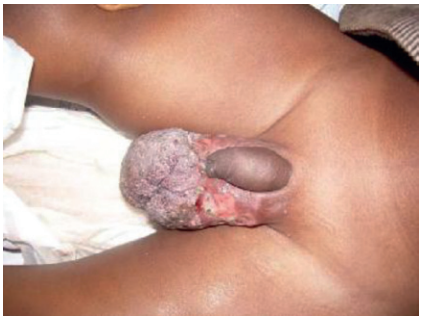


Fig. 2 **Severe scrotal condylomata accuminata.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

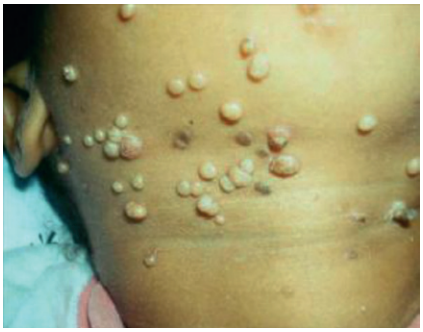


Fig. 3 **Molluscum contagiosum.** (Photo courtesy of Prof HF Jordaan, University of Stellenbosch.)

Clinical features

Condylomata accuminata (Fig. 2) are caused by HPV-6, 11 and 16, while those with malignant potential (HPV-16 and 18 being the most common) cause pre-malignant lesions that may or may not progress to malignancy in the form of penile, anal, vaginal, vulva or cervical carcinoma. Not everyone who is infected with one of these types develops malignancy, however, and there are other factors that involved in the carcinogenesis.

Management

Prevention is essential. Condoms help prevent infection in women; in men there is uncertainty. HPV vaccines are currently in clinical trials; these may prevent primary infection, but may also serve to prevent malignant change.

Condylomata accuminata can be treated with local podophyllin, cryotherapy or by surgical removal, e.g. circumcision of males if the warts are limited to the foreskin. If a small infected area remains after surgery, the warts can recur.

Cryotherapy, laser evaporation, electrodiathermy and loop excision can be used for early cervical lesions. Interferon has also been used, usually after other forms of destruction or surgery, with variable success.

For later premalignant lesions and malignant lesions, varying degrees of hysterectomy are performed in women; partial or total penectomy may be necessary in men.

Molluscum contagiosum

Epidemiology

Molluscum contagiosum appears worldwide, with greater prevalence in lower socioeconomic groups. Three types are known; type II possibly has a greater association with sexual transmission. Transmission can also occur through the shared use of towels, for instance, and scratching.

Clinical features

Initially the lesion appears as a small papule. The mature lesion is a waxy or pearl-like lesion, often umbilicated (Fig. 3). Usually the number of lesions is limited to fewer than 20 in immunocompetent individuals. Resolution is usually spontaneous.

Treatment

Surgical intervention can be of aesthetic value. Cryotherapy or chemical treatment may help; mild trauma may stimulate the immune response and result in a cure.

Cytomegalovirus

The extent to which cytomegalovirus (CMV) is spread sexually is debated, as it is difficult to determine how much of the 1% annual increase in seropositivity seen in inviduals from developed countries after puberty is due to oral mucosal contact (kissing) and genital mucosal contact, which is usually accompanied by oral mucosal contact. The most significant evidence for sexual transmission is found in the homosexual community.

Hepatitis C

Sexual transmission does occur, although most transmission is via unhygienic intravenous drug use habits, now that blood transfusions have become safe. About 170 million people are infected worldwide; prevalence varies significantly from country to country.

Other viruses transmitted sexually

Hepatitis A has been associated with sexual transmission in homosexual populations. HHV8, shed in saliva, has been associated with transmission by oral sexual practices. Certain adenoviruses, such as Ad2, Ad19 and Ad37, may cause cervicitis and urethritis.

Prevention

Avoiding infection by a sexually transmissable virus includes both behavioural and medical interventions.

The principle of ABC – abstinence, be faithful and condoms – is known to reduce sexually transmitted infection (STI) incidence. Having multiple sexual partners increases the risk of acquiring and transmitting these infections. The efficacy of condoms has been debated. They appear to be more protective to the woman than to the man, as they do not prevent exposure of the man to secretions and the perineum. They have been shown to protect against HIV and HSV transmission to both men and women; for HPV they appear to protect women to a greater degree than men. As other forms of prevention and treatment exist for most such conditions, there are ethical problems that make determining the efficacy of condom use a simple process. What is obvious is that condom usage should be applied to all sexual encounters – something that has been found to be difficult to attain for many individuals. Furthermore, an effort needs to be made to avoid the use of condoms from forming a false sense of security and increasing risk behaviour. Ideally they should be used in conjunction with microbicides.

Vaccine development is ongoing for HSV, CMV and HIV. Effective vaccines already exist for the prevention of HPV and HBV infection.

A variety of microbicides are in use for the prevention of sexual transmission of organisms; some controversy has arisen after discovery that such preventative measures may increase HIV transmission if they result in mucosal irritation.

It is not practical or logical for most people to take chronic medication for the prevention of STIs. One group where this principle is being researched is in the commercial sex worker population, where tenofovir, a nucleotide reverse transcriptase inhibitor, is being used as pre-exposure prophylaxis to prevent HIV infection.

Opportunistic viral infections

The problem of infections caused by organisms of low or moderately low pathogenicity is growing in importance as iatrogenic manipulation of the immune system becomes more sophisticated. Opportunistic infections have been defined as those which cause disease in those who have a compromised immune system. Disease is often ameliorated when immunosuppression is reduced or in the case of HIV where HAART is initiated and immune reconstitution occurs. In most cases infection with many of these organisms causes mild disease, but in the context of a poor immune response, virus is able to replicate unchecked and cause severe disease.

Table 1 highlights those viruses which cause disease in the immunocompetent, but which classically cause more severe disease in the immunocompromised.

Table 1 **Viruses causing disease in the immunocompetent but more severe disease in the immunocompromised**

Virus	Disease in the immunocompromised
HSV	Disseminated HSV, hepatitis, more frequent and severe reactivations
VZV	Haemorrhagic varicella, hepatitis, pancreatitis, disseminated zoster
Adenovirus	Disseminated infection, hepatitis, pneumonia
CMV	Colitis, retinitis, encephalitis
Human metapneumovirus	Fatal pneumonitis
HBV	Reactivation, faster progression
Parvovirus	Chronicity and anaemia
HCV	Faster progression
Molluscipox virus	Disseminated skin lesion
HPV	More rapid progression, less clearance

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papilloma virus; HSV, herpes simplex virus; PTLD, post transplant lymphoproliferative disorder

Human herpesvirus-8

Kaposi sarcoma (KS) was first described by Moritz Kaposi in 1872. The link between KS herpes virus, now known as human herpesvirus 8 (HHV-8), and KS was discovered by Chang *et al.* in 1994. HHV-8 is also associated with multicentric Castleman's disease and primary effusion lymphoma. In contrast to Epstein-Barr virus (EBV), HHV-8 is not ubiquitous. Serologic prevalence increases from 1% to 5% in blood donors in Europe and up to 80% in some parts of Africa. Horizontal transmission of virus by saliva is thought to be the most common route of infection, although vertical, sexual, blood-related and transplant-related transmission also occur (Fig. 1). HHV-8 belongs to the *Gammaherpesvirinae* subfamily and the *Rhadinovirus* genus. It is similar to other herpes viruses and has a capsid containing double-stranded DNA genome which measures between 170 kb and 270 kb. Polymorphisms in the TR region of the genome permit the classification of the virus into four distinct molecular variants.

KS is a nodular tumour arising most commonly from the lymphatic endothelium (and is, therefore, not a true sarcoma), usually affecting the skin, gastrointestinal or respiratory tract. It is dark purple in colour because of the intense vascularity which characterises this tumour. Different forms of KS have been described: classic, endemic and KS in immunosuppressed patients. Classic KS occurs mainly in the elderly male of Mediterranean origin. The tumour has a slow evolution and is not life threatening. The endemic HHV-8 occurs in countries with a high seroprevalence for HHV-8, e.g. Africa. It may be associated with HIV infection which is the aggressive form of the cancer involving any site in the body and potentially causing complication like haemorrhage and lymphatic obstruction. Diagnosis is histological and can be confirmed by detection of the viral LANA protein in the tumour.

Castleman's disease has two main subtypes, namely 1) hyaline vascular variant, usually unicentric, which is the most common form and usually asymptomatic, and 2) plasma cell variant, usually multicentric, which is associated with more aggressive symptomatic disease: characteristically fever, lymphadenopathy, hepatomegaly, splenomegaly, weight loss and night sweats. Primary effusions lymphoma

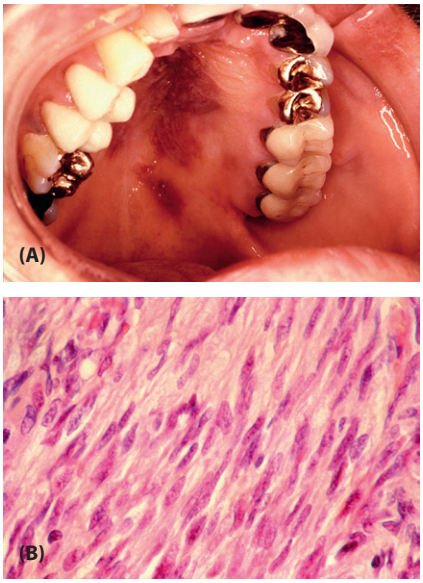


Fig. 1 **A, Karposi sarcoma (KS) of the hard palate in a HIV positive man.** (Photo courtesy of CDC/Dr Sol Silverman.) **B, Histology of KS lesion.** (Photo courtesy of CDC/Dr Steve Kraus.)

is a rare B-cell lymphoma, which develops as serous effusions in pericardial, pleural and peritoneal spaces, and is seen almost exclusively in men with HIV. Other rare associations with this virus include plasmablastic lymphoma, large cell lymphoma and peripheral T-cell lymphoma.

JC virus

JC virus belongs to the *Polyomaviridae* family of viruses. It is a neurotropic virus which only infects humans. JC virus causes progressive multifocal leukoencephalopathy (PML) (Fig. 2), a syndrome characterised by dementia, hemiparesis and disturbance of speech and vision. The virus has an almost ubiquitous distribution with a worldwide

STOP THINK

- Why do herpes viruses cause such severe disease in the immunocompromised host?

seroprevalence of about 80%, but is only activated by severe immunosuppression. PML may occur in patients on chemotherapy, AIDS patients and in patients on immunomodulatory agents for the treatment of autoimmune diseases, e.g. natalizumab. After asymptomatic infection in childhood the infection remains quiescent in the kidneys, bone marrow and lymphoid tissue. JC is a small ubiquitous DNA polyomavirus with a 5.3 kb circular enclosed DNA genome. Pathologically this virus causes demyelination, abnormal oligodendrocytes and, in the later stages of disease, severe astrocytosis. Diagnosis is usually made based on the clinical and radiological findings. Polymerase chain reaction (PCR) of CSF for JC virus may be performed, but a negative result does not exclude the diagnosis (PPV and NPV ~90%).

No specific treatment is available for the treatment of PML.

Other rare JC-associated diseases include JC virus granule cell neuronopathy, JC virus encephalopathy and JC virus meningitis.

BK

BK polyomavirus also belongs to *the Polyomaviridae* family of viruses family of DNA viruses. It has become increasingly recognised as an important cause of graft loss in kidney transplant patients. Transmission occurs in childhood likely through the respiratory or oral route. In healthy blood donors the prevalence is thought to be 82%. Once infection occurs, BK establishes latency in the renal cortex, medulla, urothelial cells and bladder. BK associated nephropathy (BKVAN) affects 1–10% of kidney-transplant patients and is primarily due to BK reactivation and replication in urothelial cells. The majority of reactivation occurs in the first year post transplant. The most common clinical manifestation is a rise in creatinine; however, ureteric stenosis and haemorrhagic cystitis can occur if the virus reactivates in the ureter or bladder respectively. Haemopoietic stem cell transplant (HSCT) patients can develop haemorrhagic cystitis due to BK virus. BK viraemia has been shown to be an independent predictor of post HCST renal impairment.

Diagnosis is made by detecting decoy cells in the urine or PCR for BK virus of blood and urine. It is well established that viruria preceeds viraemia.

Reduction in immunosuppression reduces the risk of BKVAN. Specific drug therapy is limited. Cidofovir, a nucleotide analogue of cytosine, may have some efficacy. Leflunamide is a pyrimidine synthesis inhibitor used in the treatment of rheumatic diseases, and has been shown to have antiviral properties. Other therapies that have been used include flouroquinolones, immunoglobulins and rituximab.

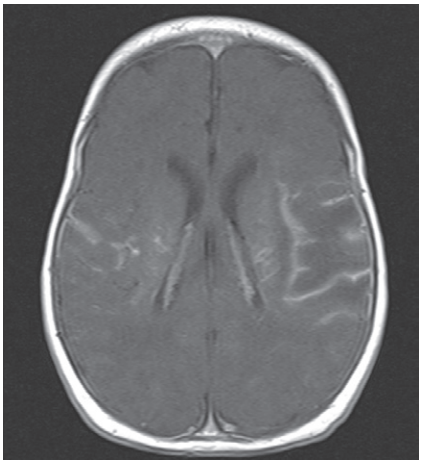


Fig. 2 **MRI scan showing demyelination classic of progressive multifocal leukoencephalopathy (PML).** (Photo courtesy of the Department of Radiology, Tygerberg Hospital, Cape Town.)

HHV-6,7

HHV-6 and 7 belong to the Betaherpesvirinae subfamily, together with CMV, but they belong to the roseolavirus genus. In comparison to the other herpes viruses they are less well understood and their clinical spectra are still being defined. Two closely related variants of HHV-6 exist, HHV-6A and HHV-6B. HHV-6A has not been linked aetiologically with any disease. HHV-6B is the agent responsible for exanthem subitum or roseola infantum, a childhood illness characterised by high fevers, skin rash and rarely complicated by encephalitis. Diagnosis of HHV-6 disease may be further complicated by the presence of integrated HHV-6 virus, which may result in very high levels of DNA. HHV-7 has been associated with some cases of exanthema subitum. Its association with pityriasis rosea remains controversial.

Studies have shown a worldwide prevalence of HHV-6 of between 70% and 95% by age 3 years. Like all herpesviruses HHV-6 and HHV-7 consist of three main structural elements: a nucleocapsid, tegument and envelope. The HHV-6 genome is a linear, double-stranded DNA molecule 160–162 kb in size. HHV-6 establishes latency in T-lymphocytes and can reactivate in response to immunosuppression. Reactivation has been described in 40% HSCT and 60% solid organ transplant patients. Reactivation occurs at a mean of 3–6 weeks post-transplant and has been associated with various clinical syndromes including fever, encephalitis, pneumonitis, hepatitis, bone marrow suppression and rash. Therapy for HHV-6 is not well established but intravenous ganciclovir, foscarnet, cidofovir have all been used in conjunction with immunoglobulin with varying success.

Key points

- Viruses which cause mild disease in the immunocompetent, may cause severe disease in those who are immunocompromised.
- Herpes viruses are an important cause of opportunistic infections.
- John Cunningham (JC) and BK viruses belong to the polyoma family and cause disease in the severely immunosuppressed.
- JC and BK viruses may both affect the kidney, whilst JC may also cause progressive multifocal leukoencephalopathy (PML) a demyelinating disease of the brain.

Eradication of viral diseases

The concept of eradication of infectious diseases emerged during the early 20th century, when a programme targeting yellow fever for eradication failed, mainly due to the existence of an animal reservoir for the virus. Efforts to eradicate malaria and smallpox followed in the mid-20th century, initiated by the World Health Organization (WHO). Malaria eradication failed, but smallpox became the first infectious disease eradicated from the globe in 1978, followed in 2010 by Rinderpest, an animal disease with high mortality and economic burdens, and caused by a morbillivirus. Smallpox eradication paved the way for strategies to eradicate other diseases. The world's attention is currently focused on efforts to eradicate wild poliovirus and efforts to eradicate measles virus were initiated in the American region in 1994 (Box 1).

Smallpox

The last natural case of smallpox in the world occurred in 1977 in Somalia (Fig. 1). Smallpox was officially declared eradicated by the WHO in 1980. The intensified eradication programme only lasted 10 years. Smallpox complied with all the criteria favouring eradication and at that time the world was relatively free from political unrest and wars. Smallpox may have been the 'ideal' disease to eradicate due to various reasons, and some authors find the application of the smallpox experience to polio eradication unrealistic. Currently, smallpox virus stocks are officially only held in two laboratories: one in the United States, the other in Russia. Recently questions arose with regards to the possibility of reintroducing smallpox as an agent in bioterrorism activities.

Polio (Fig. 2)

A programme for polio eradication was instigated in 1988. Since then polio has been eliminated from three global regions (the Americas, Western Pacific and European region). In 1988 polio was still endemic in more than 125 countries across the globe. In March 2006 the only remaining polio-endemic countries were: Nigeria, India, Afghanistan and Pakistan. Three of these countries were also the last to eliminate smallpox in the past. The reasons for the difficulties experienced with the eradication programme in these countries include: the relative inaccessibility of large and remote areas, high population densities and poverty.

Box 1 **Preconditions for eradication of an infectious disease** *(as outlined by the 1997 Dahlem Conference on Disease Eradication)*

1. No animal reservoir for the virus is known or suspected.
2. Sensitive and specific tools are available for diagnosis and surveillance.
3. Transmission from one individual to another can be interrupted.
4. Non-lethal infection or vaccination confers life-long immunity.
5. The burden of disease is important to international public health.
6. Political commitment to eradication efforts exists.

From Knobler S, Lederberg J, Pray LA (eds) Considerations for viral disease eradication: lessons learned and future strategies: workshop summary. Washington D.C.: National Academy Press, 2002.

The polio eradication programme includes four strategies. The goal of routine immunisation is to deliver four doses of vaccine to all children under the age of 1 year. Mass vaccination campaigns (national immunisation days) target all children under the age of 5 years, regardless of their previous vaccination status. These campaigns ensure that all

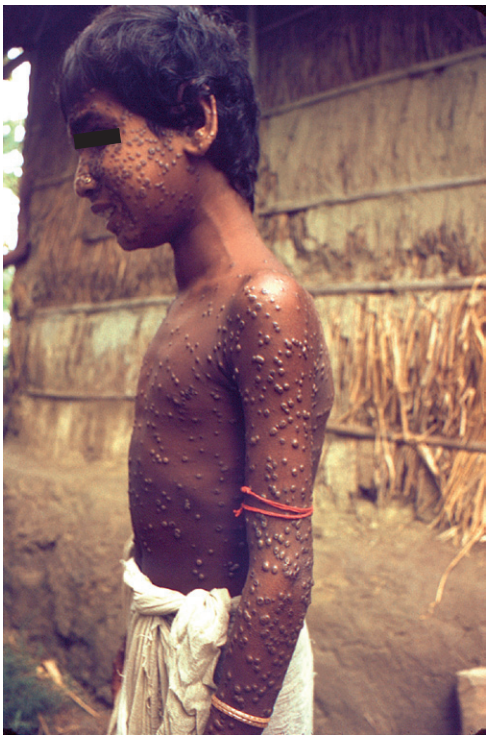


Fig. 1 **Boy with smallpox lesions.** (Photo courtesy of CDC/Jean Roy.)

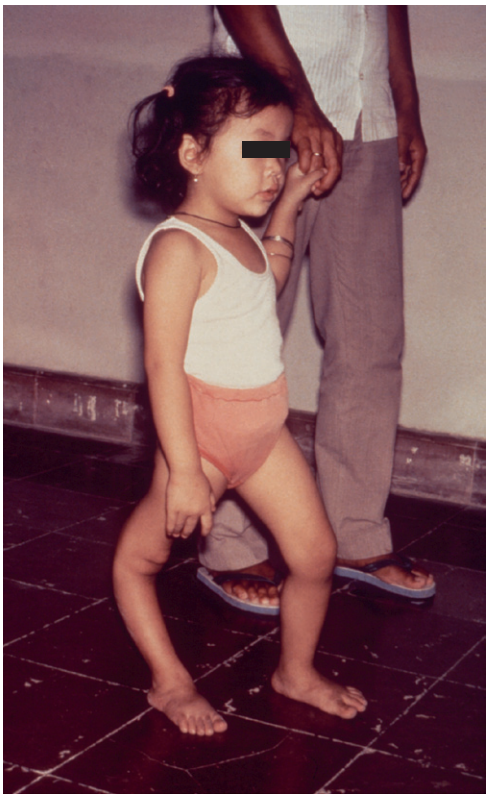


Fig. 2 **Child with a deformity in the right leg owing to polio.** (Photo courtesy of CDC.)

Post-eradication challenges

- We have to ensure that laboratory preserved virus stocks and vaccine strains of eradicated viruses are clearly labelled and securely contained within only a few reference laboratories in the world.
- Are the eradicated viruses really gone? In the era of improved molecular techniques, small viral genomes can be synthesised and converted to infectious forms.
- What are the potential problems with the use of vaccines in the post-eradication phase?
  Oral Sabin vaccine may revert back to virulent poliovirus and give rise to vaccine-associated paralytic polio (VAPP). Live attenuated vaccine strains may also be excreted for extended time periods in immunocompromised individuals.
- Bioterrorism implications.

children with no or partial immunity are vaccinated and that previously vaccinated children's immunity are boosted. Acute flaccid paralysis (AFP) surveillance aims to detect and report all children under the age of 15 years with AFP and to investigate them virologically for the presence of poliovirus in stool samples. Mop-up campaigns target areas where wild poliovirus continues to circulate. 'Door-to-door' immunisation takes place in these areas where routine immunisation coverage is usually poor, wars are raging and where restricted access to health-care services is available.

Obstacles experienced with polio eradication include political unrest, a lack of commitment and motivation by authorities, and the lack of resources, appropriate infrastructure, organisation and training of personnel involved in the programme. Attention may also be focused on more prevalent infectious diseases in Africa especially, HIV, TB and malaria. Polio eradication may not be the highest priority at present. Most cases of polio disease present asymptomatically and may not be detected by means of surveillance and diagnostic methods. Rumours about the safety of the oral Sabin vaccine (OPV) resulted in a period of poor immunisation coverage in Nigeria. Warm, tropical African climates, together with overcrowding and a lack of basic hygiene, also resulted in decreased effectiveness of the oral vaccine. Campaign fatigue was experienced in India and Nigeria during the second round of mass vaccination campaigns.

Importations of wild poliovirus into previously polio-free countries were seen in numerous African countries during the past few years (Box 2). Recombination of poliovirus with other enteroviruses, e.g. Coxsackie A, could represent a theoretical risk of reintroducing poliovirus after global transmission has ceased.

Measles

The epidemiological features of measles resemble that of polio and smallpox, although the transmission rate of measles virus is considerable higher. WHO recommends that measles elimination from a specific region only be undertaken once wild polio transmission has been interrupted and certified in that region. In the American region, measles circulation has been successfully interrupted since 2003. The measles elimination strategy used includes catch-up, keep-up and follow-up immunisation campaigns, as well as mop-up campaigns in areas where inadequate vaccination coverage is noted. Catch-up mass campaigns target all children under the age of 14 years, regardless of their previous vaccination status, and a goal of exceeding coverage of 95% is set. Keep-up campaigns represent routine immunisation practices where one dose of vaccine is administered to all infants older than 1 year. By administering measles vaccine as part of the MMR (measles, mumps and rubella) vaccine, rubella elimination may simultaneously be attempted. Follow-up campaigns should take place at time intervals of 3–5 years and during these campaigns all children above the age of 9 months who were born after the previous catch-up campaign should be immunised.

The immunisation campaigns however need to be supplemented by enhanced surveillance strategies and adequate case management, as is the case with polio.

Possible obstacles with attempts to eradicate measles may include the risk of reintroducing virus in a susceptible population due to importation, the safety of delivering vaccines through needles and syringes and the lack of research done on the effect of complications, due to both natural infection as well as live vaccine, on the ever-increasing global HIV positive population.

Conclusion

Although eradication strategies have come a long way and provide a great deal of benefit, the current delay in polio eradication and the uncertain feasibility of measles eradication demand that we re-examine the definition and justifiability of global eradication of infectious diseases. Perhaps the time has come for global strategies to aim for effective control of an infectious disease rather than global eradication.

Box 2 **The Namibian polio outbreak 2006**

Namibia experienced a polio outbreak in May–June 2006 after it had been polio-free since 1995. The dreaded disease resulted in 199 cases of acute flaccid paralysis and left 23 people dead (July 2006). The index case was a 39-year-old man from Aranos, 450 km by road from the capital, Windhoek. He underwent a cholecystectomy on 27 April in a Windhoek hospital and initially recovered well. He however, fell ill with non-specific symptoms including fever, rigors and abdominal pain and presented with paralysis of his legs and dyspnoea on 8 May 2006. Subsequently intubation and ventilation followed. Wild-type poliovirus 1 was cultured from his stool specimens. Genetic characterisation showed that the virus was closely related to type 1 polioviruses isolated in the Benguela province of Angola in May and June 2005 and that it has originated in India, one of the remaining four countries where the wild type virus is still endemic.

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