8 Viruses as Human Pathogen

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DNA Viruses

Viruses with Single-Stranded DNA Genomes

The groups of viruses with single-stranded DNA genomes are contained in only one family, the parvoviruses, with only a single human pathogen type. The *Geminiviridae*, *Circoviridae*, and many other families have circular single-stranded DNA, but infect only plants and, more rarely, animals.

Parvoviruses

■ This group's only human pathogen, parvovirus B19, is the causative virus in erythema infectiosum (also known as "slapped cheek syndrome" or the "fifth disease") in children and causes aplastic crisis in anemic patients. The virus also contributes to joint diseases, embryopathies, and tissue rejection following renal transplants. Diagnosis: serological (IgG and IgM) and PCR. ■

Pathogen. The parvoviruses are among the smallest viruses with a diameter of 19–25 mm. They are icosahedral, nonenveloped, and their genome is in the form of single-stranded DNA (ssDNA). Some parvoviruses can only replicate in the presence of a helper virus (adenovirus or herpesvirus). Parvovirus B19, the only human pathogenic parvovirus identified to date, is capable of autonomic replication, i.e., it requires no helper virus. Some zoopathic strains also show this capability in rodents, dogs, and pigs.

Pathogenesis and clinical picture. Parvovirus B19 replicates in the bone marrow in erythrocyte precursor cells, which are destroyed in the process. In patients already suffering from anemia (sickle-cell anemia, chronic hemolytic anemia), such infections result in so-called aplastic crises in which the lack of erythrocyte resupply leads to a critical shortage. In otherwise healthy persons, these infections usually run an asymptomatic course. They can, however, also cause a harmless epidemic infection in children, erythema infec-

tiosum ("slapped-cheek syndrome" or "fifth disease"). This childhood disease, which used to be classified as atypical measles, is characterized by sudden onset of exanthem on the face and extremities. Certain forms of arthritis are considered complications of a parvovirus B19 infection. The virus also appears to cause spontaneous abortions in early pregnancy and fetal damage in late pregnancy (hydrops fetalis).

Diagnosis. An enzyme immunoassay reveals antibodies of the IgG and IgM classes. During the viremic phase, at the onset of clinical symptoms, the virus can also be identified in the blood by means of electron microscopy or PCR. In-vitro culturing of the pathogen is not standard procedure.

Epidemiology and prevention. The transmission route of human parvovirus B19 is not known. Droplet infection or the fecal-oral route, analogous to other parvoviruses, is suspected. Blood and blood products are infectious, so that multiple transfusion patients and drug addicts are high incidence groups. No specific prophylactic measures are recommended.

Viruses with Double-Stranded DNA Genomes

Viruses with double-stranded DNA genomes are classified in six families: papillomavirus, polyomavirus, adenovirus, herpesvirus, poxvirus, and hepadnavirus. Carcinogenic types have been found in all groups except the poxviruses (see Chapter 7, DNA tumor viruses).

Papillomaviruses

■ The over 70 viral types in the genus ***Papillomavirus*** are all involved in the etiology of benign tumors such as warts and papillomas, as well as malignancies, the latter mainly in the genital area (cervical carcinoma). These organisms cannot be grown in cultures. Diagnosis therefore involves direct detection of the viral genome and histological analysis. Serology is less important in this group. ■

Pathogens. The papillomaviruses have a diameter of 55 nm and contain an 8 kbp dsDNA genome. There are two distinct regions within the circular genome: one that codes for the regulator proteins produced early in the replication cycle and another that codes for the structural proteins synthesized later. Over 70 papillomavirus types have been described to date, all of which induce either benign or malignant tumors in natural or experimental hosts.

Pathogenesis and clinical picture. Papillomaviruses infect cells in the outer layers of the skin and mucosa and cause various types of warts by means of local cell proliferation (Fig. 8.**1**). Specific virus types correlate with specific pathohistological wart types. Plantar and vulgar warts, flat juvenile warts, and juvenile laryngeal papillomas apparently always remain benign. By contrast, the genital warts caused by types 6 and 11 (condylomata acuminata) can show carcinomatous changes. Of all papillomavirus-caused cervical dysplasias, 50% contain human papillomavirus (HPV) 16 and 20% HPV 18.

All wart viruses induce primary proliferation of the affected cells with large numbers of viruses found in the cell nuclei. Whether a malignant degeneration will take place depends on the cell and virus type involved, but likely on the presence of cocarcinogens as well. In carcinomas, the viral DNA is found in integrated form within the host-cell genome, whereas in premalignant changes the viral genomes are found in the episomal state. Papillomaviruses possess oncogenes (*E5*, *E6*, and *E7* genes) that bind the products of tumor suppressor genes: E6 binds the *p53* gene product, E7 the *Rb* gene product (see p. 396f.).

Diagnosis. Human papillomaviruses cannot be cultivated in vitro. They are detected and identified by means of histological analysis and, in malignancies in particular, by means of in-situ hybridization. Antibody assay results have a low significance level and these procedures are not standard routine.

Epidemiology and prevention. Since viruses are produced and accumulate in wart tissues, papillomaviruses are transmissible by direct contact. Warts can also spread from one part of the body to another (autoinoculation). A certain level of prophylactic protection can be achieved with hygienic measures.

Warts Caused by Papillomaviruses

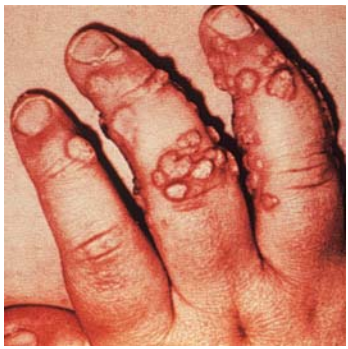

Fig. 8.**1**

Polyomaviruses

■ A medically important polyomavirus, the JC virus, causes progressive multifocal leukoencephalopathy (PML), a demyelinating disease that has become more frequent as a sequel to HIV infections, but is otherwise rare. The same applies to the BK virus, which affects bone marrow transplantation patients. Electron microscopy or PCR are the main diagnostic tools. ■

Pathogens. The polyomaviruses can be divided into two groups: in one group are the SV40 and SV40-like viruses (Fig. 8.**2**) such as human pathogen JC and BK viruses. In the other are the true polyomaviruses such as the carcinogenic murine polyomavirus. The designations JC and BK are the initials of the first patients in whom these viral types were identified. There are also a number of other zoopathic oncogenic polyomaviruses. The name *polyoma* refers to the ability of this organism to produce tumors in many different organs.

Pathogenesis and clinical picture. The JC and BK viruses are widespread: over 80% of the adult population show antibodies to them, despite which, clinical manifestations like PML are very rare. The viruses can be reactivated by a weakening of the immune defense system. The JC virus attacks the macroglia, especially in AIDS patients, to cause progressive multifocal PML, a demyelinating process in the brain with disseminated foci that is fatal

Polyomaviruses (SV40)

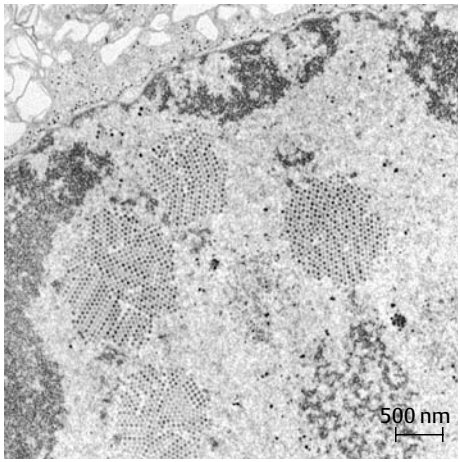

Fig. 8.**2** Section through viral conglomerations in the nucleus of the host cell (TEM).

within one year. The BK virus can cause hemorrhagic cystitis in bone marrow transplantation patients.

Diagnosis. The JC and BK viruses can be grown in cultures, albeit with great difficulty and not for diagnostic purposes. Both can be detected with PCR and the BK virus can be seen under the electron microscope in urine. Antibody assays are practically useless due to the high level of generalized contamination.

Epidemiology. Despite the high level of generalized contamination, the transmission routes used by the human polyomaviruses have not been clarified.

Adenoviruses

There are a total of 41 types of adenoviruses and they cause a wide variety of diseases. Influenza infections of the upper, less frequently the lower, respiratory tract and eye infections (follicular conjunctivitis, keratoconjunctivitis) are among the more significant clinical pictures. Intestinal infections are mainly caused by the only not culturable virus types 40 and 41. Diagnosis: antibody assay in respiratory adenovirus infections. Serology is not reliable in the eye and intestinal infections. It is possible to isolate the pathogens in cell cultures from eye infections. Enteral adenoviruses are detected in stool by means of electron microscopy, enzyme immunoassay, or passive agglutination.

Pathogens. Adenoviruses are nonenveloped, 70–90 nm in size, and icosahedral. Their morphogenesis occurs in the cell nucleus, where they also aggregate to form large crystals (Fig. 8.**3**). Their genome is a linear, 36–38 kbp double-stranded DNA. Adenoviruses got their name from the adenoidal tissues (tonsils) in which they were first identified.

Pathogenesis and clinical picture. Adenoviruses cause a variety of diseases, which may occur singly or concurrently. The most important are infections of the upper (sometimes lower) respiratory tracts, the eyes, and the intestinal tract.

Infections of the **respiratory tract** take the form of rhinitis or abacterial pharyngitis, depending on the virus type as well as presumably on the disposition of the patient. They may also develop into acute, influenzalike infections or even, especially in small children, into a potentially fatal pneumonia.

The **eye infections**, which may occur alone but are often concurrent with pharyngitis, range from follicular conjunctivitis to a form of keratoconjunctivitis that may even cause permanent partial loss of eyesight.

Adenoviruses

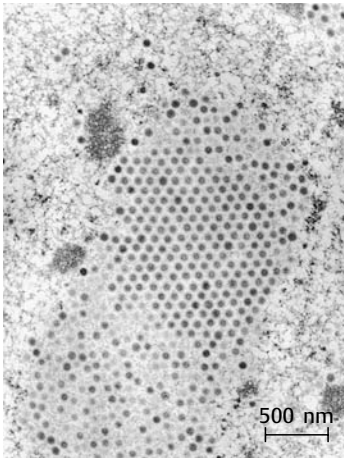

Fig. 8.**3** Viral crystals in the nucleus of the host cell (TEM).

An important aspect of the **intestinal infections** is that the primary gastroenteritis forms are caused by the viral strains 40 and 41, which are difficult to culture.

Adenoviruses can persist for months in the regional lymph nodes or tonsils until they are reactivated.

Diagnosis. Antibody assays in patient serum are the main approach taken in respiratory adenovirus infections. Serology is unreliable in the eye and intestinal infections, since hardly any antibodies are produced in response to such highly localized infections. It is possible to isolate the viruses that cause respiratory infections by inoculating cell cultures with pharyngeal material or bronchial secretion and with conjunctival smears in eye infections. Enteral adenoviruses, on the other hand, are hard to culture. The best approach to detecting them is therefore to subject stool specimens to electron microscopy, enzyme immunoassay, or passive agglutination methods.

Epidemiology and prevention. Humans are the source of infection. Susceptibility is the rule. Generalized contamination of the population begins so early in childhood that adenovirus infections play a more significant role in children than in adults. Transmission of respiratory adenoviruses is primarily by droplet infection, but also as smear infections since the virus is also excreted in stool. Eye infections can be contracted from bathing water or, in the case of adenovirus type 8 in particular, iatrogenically from insuffi-

ciently sterilized ophthalmological instruments. The enteral infections are also transmitted by the fecal-oral route, mainly by contact rather than in water or food. Adenoviruses are the second most frequent diarrhea pathogen in children after rotaviruses (p. 456f.).

Herpesviruses

The viruses in this family all feature a practically identical morphology, but show little uniformity when it comes to their biology and the clinical pictures resulting from infections. One thing shared by all herpesviruses is the ability to reactivate after a period of latency.

The **herpes simplex virus** (HSV, two serotypes) is the pathogen that causes a vesicular exanthem (fever blisters, herpes labialis, or genitalis), encephalitis, and a generalized infection in newborns (herpes neonatorum).

The **varicella-zoster virus** (VZV) causes the primary infection chickenpox, which can then recidivate as zoster (shingles).

Cytomegalovirus (CMV) infections remain inapparent or harmless in the immunologically healthy, but can cause generalized, fatal infections in immunocompromised individuals.

The **Epstein-Barr virus** (EBV) is the pathogen in infectious mononucleosis and is also implicated in lymphomas (including Burkitt lymphoma) and nasopharyngeal carcinomas.

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Human herpesvirus 6 (HHV 6) is the pathogen that causes three-day fever (exanthema subitum, roseola infantum).

Human herpesvirus 8 (HHV 8) causes the AIDS-associated Kaposi sarcoma.

Diagnosis. Isolation, amplification culture, or direct detection can be used to diagnose herpes simplex, varicella-zoster, and cytomegaloviruses; antibody assays can be used for Epstein-Barr, human herpes 6 and 8, and varicella-zoster viruses; PCR can detect herpes simplex, varicella-zoster virus, cytomegalovirus, and human herpesvirus 6.

Therapy. Effective and well-tolerated chemotherapeutics are available to treat herpes simplex, varicella-zoster virus, and cytomegalovirus (acyclovir, ganciclovir).

Biology of the Herpesviruses

Several hundred herpesvirus species have been described in humans and animals, all with the same morphology (Fig. 8.**4a**). They have dsDNA genomes. Replication of the DNA and the morphogenesis of the virus particle take place in the host-cell nucleus. The envelope (inner nuclear membrane) is then formed when the virus penetrates the nuclear membrane (Fig. 8.**4b**), whereby depending on the cell and viral type involved a more or less substantial number of viruses receive an envelope after reaching the cytoplasm, at the cell membrane or not at all. The envelope is the major determinant of viral infectivity (see Chapter 7, p. 378f.). Since the envelope contains mainly host-cell determinants, it can also be assumed that it provides a level of protection from host immune responses.

Common to all herpesviruses is a high level of generalized contamination (60–90% carriers) and the ability to persist in a latent state in the body over long periods. The different viral species persist in different cells, whereby the cell type is the decisive factor determining latency or replication of the virus. Herpes simplex virus and varicella-zoster virus do not produce any virus particles during latency, although they do produce one, or a few, mRNA types and the corresponding proteins. Cytomegalovirus and Epstein-Barr virus appear to maintain continuous production of small numbers of viruses as well, so that fresh infection of a small number of new cells is an ongoing process. These viruses would appear to produce persistent, subclinical infections concurrently with their latent status (p. 394). Reactivation of these latent viruses is apparently initiated by a number of factors (psychological stress, solar irradiation, fever, traumata, other infections, immunosuppressive therapy), but the actual mechanisms that reactivate the lytic viral life cycle are unknown.

Human herpesviruses (with the exception of the varicella-zoster virus) and many zoopathic herpes species have also been implicated in the etiology of malignancies.

Eight human herpesviruses that infect different organs are known to date, e.g., the skin (herpes simplex virus types 1 and 2, varicella-zoster virus), the lymphatic system (Epstein-Barr virus, human herpesvirus type 6, cytomegaloviruses), and the CNS (herpes simplex virus, cytomegalovirus).

Herpes simplex Virus (HSV)

Pathogen, pathogenesis, and clinical picture. The viral genome codes for about 90 proteins, categorized as "immediate early" (regulatory functions), "early" (DNA synthesis), and "late" (structural) proteins. Herpes simplex viruses are classified in types 1 and 2, which differ both serologically and biologically (host-cell spectrum, replication temperature). Initial infection with **herpes simplex type 1** usually occurs in early childhood. The portal of entry is normally the oral mucosa ("oral type") and the infection usually manifests as a gingivostomatitis. The viruses then wander along axons into the CNS, where they persist in a latent state in the trigeminal (Gasseri) gang-

Herpesviruses

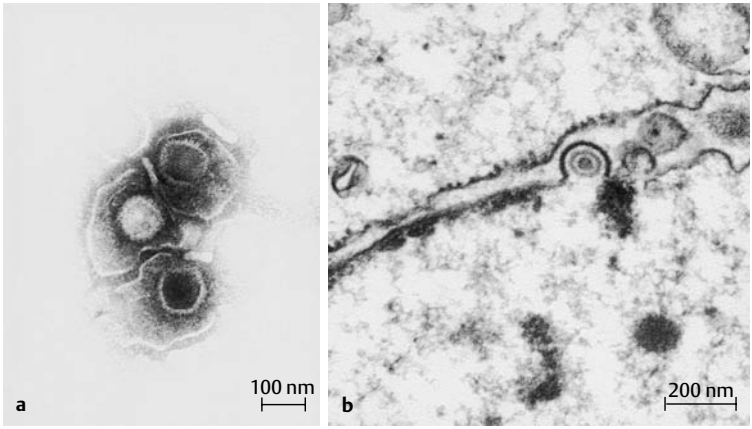

Fig. 8.**4** **a** With envelope, **b** in the nucleus of the host cell; envelope formation at the nuclear membrane. The naked virion measures 100 nm and the virus with its envelope up to 200 nm.

lion. As with all herpesviruses, the pathogen remains in the macroorganism *permanently* after the primary infection. Following reactivation (endogenous recidivation), the viruses follow the same route back to the periphery, where they cause the familiar vesicular exanthem ("fever blisters," herpes labialis, Fig. 8.**5**). Despite established immunity, such recidivations can manifest repeatedly because the viruses wander within the nerve cells and do not enter

Herpes labialis

Fig. 8.**5** Following the initial infection, herpes simplex viruses (HSV) persist in the latent state in nerve cells of the CNS. When reactivated, they travel down the axons of these cells to the periphery, where they cause the typical vesicular exanthem.

the intercellular space, thus remaining beyond the reach of the immune defenses. Possible complications include keratoconjunctivitis and a highly lethal form of encephalitis.

The initial infection with **HSV type 2** normally affects the urogenital area ("genital type") and can be contracted despite an existing HSV type 1 infection. HSV type 2 persists in the latent state in the lumbosacral ganglia or peripheral tissues, from where it causes episodes of manifest herpes genitalis. Neurological complications are very rare and more benign than in HSV type 1. On the other hand, infections of newborn children (herpes neonatorum), e.g., in cases of maternal genital herpes, are feared for their high lethality rate.

Diagnosis. Cultivating the pathogen from pustule contents is the method of choice in labial and genital herpes. In an HSV encephalitis, the cerebrospinal fluid will contain few viruses or none at all. In such cases, they can only be cultivated from tissues (biopsy or autopsy material). Virus detection by means of cerebrospinal fluid PCR is worth a try.

Direct detection of the viruses under an electron microscope is only practicable if the specimen contains large numbers of viruses, which in practice will normally only be the case in blister contents. The virus can also be detected directly in patient specimens using immunofluorescence or in-situ hybridization (p. 408), but the material must contain virus-infected cells, i.e., blister contents are not as suitable here as in electron microscopy and virus isolation.

Serological investigation results in HSV lack significance due to the high level of general contamination in the population.

Epidemiology, prevention, and therapy. HSV type 1 is transmitted by contact, and possibly by smear infection as well. Contamination with HSV therefore begins in early childhood. Transmission of HSV type 2 usually occurs during sexual intercourse, so that infections are generally not observed until after puberty. No immune prophylaxis (vaccination) is currently available for HSV. Acycloguanosine is used prophylactically in immunosuppressed patients (see Chapter 7, p. 404).

Specific therapy is possible with acycloguanosine. Used in time, this chemotherapy can save lives in HSV encephalitis.

Varicella-zoster Virus (VZV)

Pathogen, pathogenesis, clinical picture. The VZ virus differs substantially from HSV, both serologically and in many biological traits. For instance, it can only be grown in primate cell cultures, in which it grows much more slowly and more cell-associated than is the case with HSV. No subtypes have been described.

Herpes zoster

Fig. 8.**6** The varicella zoster viruses (VZV) persist in the latent state in spinal ganglia cells. When reactivated, they cause dermal efflorescences in the corresponding dermatome.

The initial infection with VZV manifests in the great majority of persons as chickenpox, an episodic papulous exanthem. The portals of entry are the nasopharyngeal space and the conjunctiva. From there, the virus undergoes a viremic phase in which it is transported by the blood to the skin, where the typical exanthem is produced. The disease confers an effective immunity. In immunodeficient patients, a VZV infection (or reactivation, see below) can affect other organs (lungs, brain) and manifest a severe, frequently lethal, course.

After the symptoms of chickenpox have abated, the VZV persists in the spinal ganglia and perhaps in other tissues as well. Following reactivation, zoster (shingles) develops (Fig. 8.**6**), whereby the virus once again spreads neurogenically and causes neuralgia as well as the typical zoster efflorescence in the skin segment supplied by the sensitive nerves. Reactivation is induced by internal or external influences and becomes possible when cellular VZV immunity drops off, i.e., after about the age of 45 assuming normal immune defenses.

Diagnosis. VZV can be detected with a wide spectrum of methods, namely PCR, isolation, direct viral detection by means of electron microscopy, detection of viral antigens using immunofluorescence in tissue specimens or cell smears, and serologically based on antibody titer increases or IgM detection.

Epidemiology, prevention, and therapy. VZV is highly contagious and is transmitted aerogenically. The primary infection, which manifests as chickenpox, is still almost exclusively a childhood disease today. A vaccine containing attenuated viruses is available for prevention of chickenpox and possibly zoster, but its use is currently a matter of controversy. In immunosuppressed patients, hyperimmunoglobulin can be used for passive immunization or postexposure immunity. Acycloguanosine is used both prophylactically and in treatment of VZV infections.

Cytomegalovirus (CMV)

Pathogen, pathogenesis, clinical picture. CMV is characterized by a narrow spectrum of hosts, slow replication, frequently involving formation of giant cells and late, slow development of cytopathology.

An initial infection with cytomegaly is inapparent in most persons, even in very early—perinatal or postnatal—infections. The virus apparently persists in the latent state in mononuclear cells. Reactivation can also run an asymptomatic course, but symptoms may also develop that are generally relatively mild, such a mononucleosislike clinical pictures, mild forms of hepatitis or other febrile illnesses. Droplet infection is the most frequent route of transmission, but smear infections and nursing infections are also possible. Generalized contamination with this pathogen (over 90% of the adult population is infected), frequent reactivation with, in some cases, months of continued excretion of viruses in saliva and urine and the wide variety of potential clinical pictures are all factors that often make it difficult to implicate CMV as the etiological cause of an observed illness. The virus infection can manifest as a sequel instead of a cause, for instance of a flulike illness. To labor the point somewhat, it could be said that the patient is not primarily ill due to a CMV infection, but rather has a florid CMV infection because he or she is ill.

The situation is different in AIDS, transplantation or malignancy patients, in whom a fresh CMV infection or reactivation—similarly to HSV and VZV—can result in severe generalized infections with lethal outcome. The liver and lungs are the main organs involved. Retinitis is also frequent in AIDS patients. In kidney transplant patients, a CMV infection of the mesangial cells can result in rejection of the transplant. Another feared CMV-caused condition is an intrauterine fetal infection, which almost always results from a primary infection in the mother: in 10% of cases the infection results in severe deformities.

Diagnosis. Amplification cultures (p. 408f.) from saliva, urine, buffy coat, tissue, or BAL (bronchoalveolar lavage) are a suitable method of confirming a florid CMV infection. In transplantation patients, the risk of a CMV manifestation can be estimated by immunocytochemical monitoring of the CMV-positive cell count in the peripheral blood ("antigenemia test"), since this count normally rises several days before clinical manifestations appear. Based on such an early warning, antiviral therapy can be started in time (ganciclovir, foscarnet). PCR results must be interpreted with a clear idea of how sensitive the method used can be, since the numbers of viruses found may be clinically insignificant. Hasty conclusions can result in "overdiagnosis," above all in CMV-positive transplant recipients.

Serological results are hardly useful in clarifying a florid infection due to the high level of generalized contamination. Added to this is the fact that the immunoincompetent patients in whom diagnosis of this infection would

be particularly important are serologically problematical anyway. Serology does contribute to clearing up the CMV status of transplant recipients and donors.

Epidemiology, prevention, and therapy. CMV is transmitted by contact or smear infection, usually in childhood or adolescence. Immunosuppressed patients can be treated with hyperimmunoglobulin to provide passive immunity against infection or recidivation. Ganciclovir and foscarnet are therapeutically useful in transplantation, and particularly in AIDS patients, to combat CMV-induced pneumonia, encephalitis, and retinitis.

Epstein-Barr Virus (EBV)

Pathogen, pathogenesis, clinical picture. EBV infects only a narrow spectrum of hosts and replicates very slowly. It persists in a latent state in B lymphocytes and can lead to their immortalization and tumor transformation.

EBV enters the body through the mucosa. It replicates in epithelial cells of the oropharynx or cervix and enters B lymphocytes, where it continues to replicate. This results in the clinical picture of infectious mononucleosis (kissing disease or Pfeiffer disease), which is characterized by fever and a generalized but mainly cervical swelling of the lymph nodes, typically accompanied by tonsillitis, pharyngitis, and some cases of mild hepatic involvement. This virus also persists in latency, probably for the life of the patient, in (immortalized) B cells.

EBV and EBV-specific sequences and antigens are isolated in cases of Burkitt lymphoma and nasopharyngeal carcinoma. The higher incidence of Burkitt lymphoma in parts of Africa is attributed to a cofactor arising from the hyperendemic presence of malaria there. EBV exacerbates the B-cell proliferation resulting from a malaria infection. EBV has also been implicated in Hodgkin disease and T-cell lymphomas. These tumor forms also result from the interaction of EBV with other mechanisms of cell damage. In immunocompetent persons, the following lymphoproliferative diseases are sequelae of EBV infections:

- a benign polyclonal B-cell hyperplasia,
- its malignant transformation into a polyclonal B-cell lymphoma, and
- a malignant, oligoclonal or monoclonal B-cell lymphoma.

Diagnosis. Heterogenetic antibodies that agglutinate the erythrocytes of several animal species and antibodies to a variety of viral antigens are found in **acute** infectious mononucleosis:

- **VCA** (viral capsid antigen). Antibodies to VCA appear early and persist for life.
- **EA** (early antigen). Antibodies to EA are only detectable during the active disease.

– **EBNA** (Epstein-Barr nuclear antigen). Antibodies to EBNA are not produced until two to four weeks after disease manifestation, then persist for life.

Chronic mononucleosis is characterized by antibodies to VCA and EA.

The diagnostic procedures in lymphoproliferative diseases (see above) involve histology and cellular immunotyping.

Epidemiology, prevention, and therapy. EBV is excreted in saliva and pharyngeal secretions and is transmitted by close contact ("kissing disease"). As with all herpesviruses the level of generalized contamination is high, with the process beginning in childhood and continuing throughout adolescence. Neither immunoprophylactic nor chemoprophylactic measures have been developed as yet. Lymphoproliferative diseases involving viral replication can be treated with acyclovir and ganciclovir.

Human Herpesvirus (HHV) 6

Pathogen, pathogenesis, clinical picture. HHV-6 was isolated in 1986 in patients suffering from lymphoproliferative diseases and AIDS. The virus shows T-cell tropism and is biologically related to the cytomegalovirus. HHV-6 exists in two variants, HHV-6A and HHV-6B. The pathogenic implications of their reactivation have not yet been described.

HHV-6B is the causal pathogen in exanthema subitum (roseola infantum), a disease that is nearly always harmless, characterized by sudden onset with high fever and manifests as a typical exanthem in small children. Reports of HHV-6-caused illness in adults are rare and the clinical pictures described resemble mononucleosis (EBV-negative mononucleosis). Apparently, however, this virus can also cause severe infections in bone marrow transplant patients (pulmonary and encephalitic infections). HHV-6A has not yet been convincingly implicated in any clinical disease.

Diagnosis and epidemiology. HHV-6 can be cultured in stimulated umbilical lymphocytes. Potentially useful diagnostic tools include antibody assay and PCR.

Generalized contamination with HHV 6 begins in early childhood, eventually reaching levels exceeding 90% in the adult population. The virus persists in latent form in the salivary gland, so that mother-to-child transmission is most likely to be in saliva.

Human Herpesvirus (HHV) 8

Pathogen, clinical picture. HHV 8 has recently been identified as a decisive cofactor in induction of Kaposi sarcoma. The classic, sporadic form of this malignancy was described in 1872 in the Mediterranean area. It also occurs

following organ transplantations and is a significant cause of death in AIDS patients (12%).

The contribution of HHV 8 to the pathogenesis of Kaposi sarcoma appears to lie in dysregulation of cytokine and hormone production. In transplantation-association Kaposi sarcoma the virus can also be transmitted by the transplant.

Diagnosis. Antibody assay (EIA, IF, Western blot).

Poxviruses

The variola virus, which belongs to the genus *Orthopoxvirus* and is the causative agent in smallpox, was declared eradicated in 1980 after a WHO vaccination campaign. The **vaccinia virus**, used at the end of the 18th century by E. Jenner in England as a vaccine virus to protect against smallpox, is now used as a vector in molecular biology and as a hybrid virus with determinants from other viruses in experimental vaccines. Among the other orthopoxviruses found in animals, the monkeypox viruses are the main human pathogens. The animal pathogens **parapoxviruses** (milker's nodules, orf) are occasionally transmitted to humans, in whom they cause harmless exanthems.

The molloscum contagiosum virus affects only humans and causes benign tumors.

Diagnosis. Orthopoxviruses and parapoxviruses: electron microscopy. Molloscum contagiosum: histology.

Brick-Shaped Orthopoxvirus

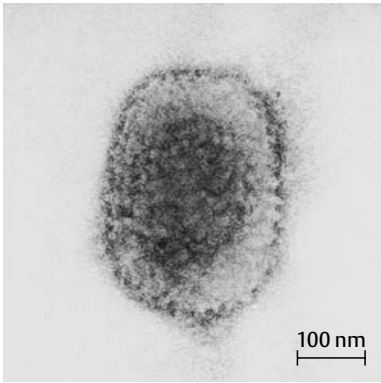

Fig. 8.**7** Poxviruses measure 200–350 nm, putting them just within the resolution range of light microscopes (TEM).

Vaccinia Viruses

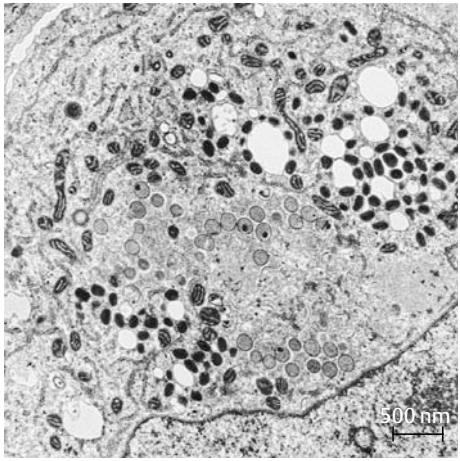

Fig. 8.**8** The vaccinia viruses are the dark, electron-dense inclusions readily visible here. They replicate in a discrete cytoplasmic region (TEM).

Pathogens. The viruses of the pox group are the largest viruses of all. At 230 × 350 nm they are just within the resolution range of light microscopes. They have a complex structure (Fig. 8.**7**) and are the only DNA viruses that replicate in a defined area within the host-cell cytoplasm, a so-called "virus factory" (Fig. 8.**8**).

The diseases smallpox (variola major) and the milder form alastrim (variola minor) now no longer occur in the human population thanks to a worldwide vaccination program during the 1970s. The last person infected by smallpox was registered in Somalia in 1977 and eradication of the disease was formally proclaimed in 1980. Since then, populations of the virus have been preserved in two special laboratories only.

The Family *Poxviridae*

This virus family comprises several genera:

- the **orthopox**viruses include the variola and alastrim viruses, the vaccinia virus used in smallpox vaccines, the closely related (but not identical) cowpox viruses as well as the monkeypox, mousepox, and rabbitpox viruses.

Other genera with human pathogen strains include:

- the **parapox**viruses including the orf virus and the milker's nodule virus (not to be confused with cowpox), transmitted to humans by sheep and cows, respectively;
- the **molluscipox**viruses, i.e., the molluscum contagiosum virus.

Pathogenesis and clinical picture. Variola viruses are transmitted aerogenically. The mucosa of the upper respiratory tract provides the portal of entry. From there, the pathogens enter the lymphoid organs and finally penetrate to the skin, where typical eruptions form and, unlike varicella pustules, all develop together through the same stages (Fig. 8.**9**). The mucosae of the respiratory and intestinal tracts are also affected. Lethality rates in cases of smallpox (variola major) were as high as 40%. In alastrim (variola minor) the level was 2%, whereby the cause of death was often a bronchopneumonia.

The vaccinia virus is a distinct viral type of unknown origins, and not an attenuated variola virus. It was formerly used as a vaccine virus to protect against smallpox. The vaccination caused a pustular exanthem around the vaccination site, usually accompanied by fever. Encephalitis, the pathogenesis of which was never completely clarified, was a feared complication. It is assumed that an autoimmune reaction was the decisive factor. Other complications include generalized vaccinia infection and vaccinial keratitis. Vaccinia infections and their complications disappeared for the most part when smallpox was eradicated by the WHO vaccination campaign. Vaccinia viruses are still frequently used as vectors in molecular biology laboratories. The inherent pathogenicity of the virus should of course be kept in mind by experimenters.

Infections with *cowpox*, *orf*, and *milker's nodule* viruses are rare and usually harmless. The lesions remain localized on the skin (contact site), accompanied by a local lymphadenitis. These are typical occupational infections (farmers, veterinarians). The *molluscum contagiosum* virus is unusual in that in-vitro culturing of the virus has not succeeded to date. Infections with this virus do not confer immunity. The infection causes epidermal, benign tumors ("molluscum contagiosum warts").

Smallpox (Variola)

Fig. 8.**9** In contrast to the lesions in varicella infections, all smallpox pustules are at the same developmental stage.

Diagnosis. The poxviruses group are relatively easy to recognize under an electron microscope in pustule contents, provided the pustules have not yet dried out or been superinfected with bacteria. Orthopoxviruses and parapoxviruses can be differentiated morphologically, but the viruses within each genus share the same morphology. Molluscum contagiosum is diagnosed histologically.

Epidemiology and prevention. Diseased humans were the sole viral reservoir of variola and alastrim. Transmission was direct and aerogenic and, although the virus is highly resistant even when desiccated, less frequent via contaminated objects (bed linens). The vaccinia virus does not occur in nature and any human infections are now accidental (laboratory infections).

The zootropic poxviruses are transmitted solely by means of contact with infected animals. Molluscum contagiosum is transmitted by interhuman contact.

Hepadnaviruses: Hepatitis B Virus and Hepatitis D Virus

■ A hepatitis B virus (HBV) infection (see p. 385ff., replication) of the liver cells results in expression of viral antigen on the cell surface, followed by immunological cell damage with acute, possibly fulminant, chronic persistent or chronic aggressive hepatitis. The final stages can be liver cirrhosis or hepatocellular carcinoma. A concurrent or later superinfection by a defective, RNA-containing and HBV-dependent hepatitis D virus (HDV, delta agent) normally exacerbates the clinical course. Both viruses are transmitted in blood or body fluids, whereby even a tiny amount of blood may be enough to cause an infection.

Diagnosis: immunological antigen or antibody assay in patient serum. The antigen or antibody patterns observed provide insights on the stage and course of the disease.

Prevention: active immunization with HBV surface (HBs) antigen; concurrent postexposure passive immunization. ■

Hepatitis B pathogen. The *hepatitis B virus* (*HBV*) is the main representative of the family of hepadnaviruses, *Hepadnaviridae*. The name of the family is an acronym of the disease caused by the virus and its genomic type. It causes a sometimes chronic form of liver inflammation (hepatitis) and its genome consists of partially double-stranded DNA (hepadnavirus = hepatitis DNA virus). The replication cycle of the HBV includes a transient RNA phase (for details see Chapter 7, p. 385). The HBV possess an envelope made up

Hepatitis B Virus

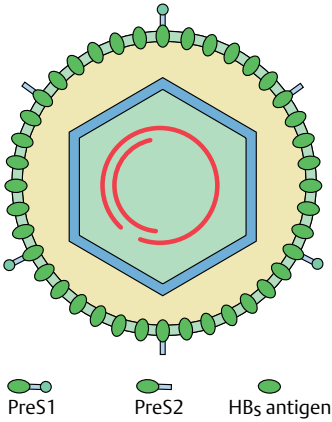

Fig. 8.**10** The capsid, which consists of Hbc and Hbe antigens, encloses the entire DNA antisense strand, the incomplete sense strand, and the reverse transcriptase (not shown here). The envelope contains the three forms of the Hbs antigen: PreS1 = complete protein, PreS2 = shortened form of PreS1, HBs antigen = HB surface antigen in the proper sense, shortened form of PreS2.

of a cellular double lipid layer in which are integrated the hepatitis B surface (HBs) antigen, a 25 kDa polypeptide, and its precursor stages PreS1 (40 kDa) and PreS2 (33 kDa). (Fig. 8.**10**). This envelope encloses the actual capsid, which consists of the hepatitis B core (HBc) antigen with 21 kDa and contains the genome together with the DNA polymerase (a reverse transcriptase, p. 385). The complete, infectious virion, also known as a Dane particle after its discoverer, has a diameter of 42 nm, the inner structure 27 nm. The virus replicates in liver cells. The Dane particles and the HBs antigen, but not the HBc antigen, are released into the bloodstream, whereby the HBs antigen is present in two different forms, a filamentous particle approximately 22 × 100 nm and a spherical form with a diameter of about 22 nm. A further viral protein is the HBe antigen, which represents a posttranslational, truncated form of the HBc antigen and is no longer capable of spontaneous capsid formation. It is also released from the hepatic cells into the blood.

Hepatitis B Mutants

Using molecular biological methods refined in recent years, more and more HBV mutants have been found with one or more amino acid exchanges in certain proteins. HBs or PreS mutants are so-called “escape” mutants that can cause a new infection or recidivation despite immune protection by antibodies to HBs. Similarly, pre-HBVc or HBVc mutants can lead to a reactivation of HBV replication and thus to a chronic hepatitis, since they block formation of the HBe antigen and thus the point of attack for the cellular immune defenses. These HBc mutants are frequently observed under interferon therapy.

Hepatitis D pathogen. A certain percentage of HBV-infected persons, which varies geographically, are also infected by a second hepatitis virus discovered at the end of the seventies in Italy, the *delta agent* or *hepatitis D virus* (*HDV*). It was originally thought to be a new HBV antigen. In fact, it is an unclassified RNA virus that codes for the delta antigen. Its capsid consists of HBs antigen, i.e., HBV-coded material. For this reason, the virus can only replicate in persons infected with HBV (in this case the "helper virus").

The delta agent is 36 nm in size and possesses a very short viral RNA containing 1683 nucleotides. This RNA is circular, has antisense (minus) polarity and is reminiscent in size and structure of the RNA in plant viroids (p. 472f.). Its transcription and replication take place in the cell nucleus by means of a cellular polymerase. The resulting RNA sense strand contains, in contrast to viroids, a protein-coding segment comprising about 800 nucleotides, which the cell processes into an mRNA. The HDV codes for two proteins with 27 and 29 kDa (delta antigen). The shorter protein with 195 amino acids, which promotes RNA replication, is produced earlier in the replication cycle. Later, after the stop codon UAG of the mRNA has been transformed (enzymatically?) into UGG, the longer protein with 214 amino acids is synthesized; it inhibits replication and controls the encapsidation of the HDV RNA in the HBs antigen.

Pathogenesis and clinical picture. The incubation period of hepatitis B is four to 12 weeks, followed by the acute infection phase, icteric, or anicteric course, once again with a variable duration of two to 12 weeks. The hepatic cell damage resulting from an HBV infection is not primarily due to cytopathic activity of the virus, but rather to a humoral and cellular immune response directed against the virus-induced membrane antigens (HBs, HBc) on the surface of the infected hepatocytes: 0.5–1% of those infected experience a fulminant, often lethal, hepatitis. In 80–90% of cases the infection runs a benign course with complete recovery and elimination of the HBV from the body. A chronic infection develops in 5–10% (see p. 393, persistent viral infections). Three forms are differentiated, but mixed forms are possible:

- healthy HBV carriers,
- chronic persistent hepatitis (CPH) without viral replication, and finally
- chronic aggressive hepatitis (CAH) with viral replication and a progressive course.

A chronic infection can result in development of a carcinoma (hepatocellular carcinoma, HCC) or cirrhosis of the liver, with incidence varying widely from one geographic area to another. The delta agent appears to have an unfavorable influence on the clinical course, usually making the disease more aggressive, increasing the number of complications and worsening the prognosis.

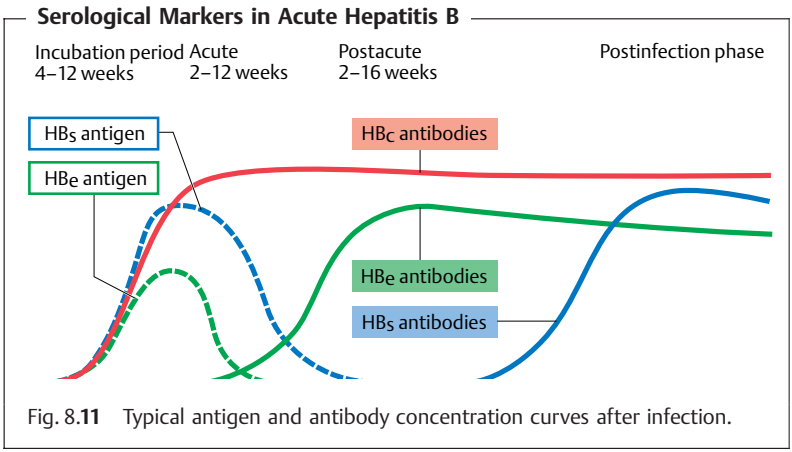

Fig. 8.**11** Typical antigen and antibody concentration curves after infection.

Diagnosis. Hepatitis B is diagnosed by identifying the various HBV antigens or the antibodies directed against them. Both antigens and antibodies can be detected in patient blood using a solid phase test (enzyme immunoassay). The individual components manifest in specific patterns. Fig. 8.**11** shows the sequence of phases in an uncomplicated hepatitis B infection, upon which the guiding principles in laboratory diagnosis of HBV infections are based (Table 8.**1**).

The hepatitis D virus is diagnosed by detection of delta antigen or possibly antibodies to delta (IgM) in the blood.

Tab. 8.**1** Laboratory Diagnostics in HBV Infections

Status	Diagnostic test
Acute infection	HBc-IgM, HBs-Ag
Vaccine immunity	HBs-IgG
Recovered, healed	HBs-IgG, HBc-IgG
Chronic, patient infectious	HBe and HBs-Ag, PCR
Exclusion of HBV	HBc-IgG negative
Serology inconclusive, mutants, therapeutic monitoring	Quantitative PCR

Chronic hepatitis B

Development of a chronic hepatitis B infection is revealed by a changed antigen-antibody profile: the two antigens HBs and HBc (and raised transaminases) persist for over six months, whereby antibodies to HBe and HBs are not produced. A subsequent "late seroconversion" of HBe antigen to anti-HBe antibodies supports a better prognosis. Thorough clarification of chronic cases must include either immunohistological testing for HBV antigens in liver biopsies or PCR testing for the presence of viral DNA, and thus Dane particles, in patient serum.

Epidemiology and prevention. Humans are the sole reservoir of HBV. Transmission is parenteral, either with blood or body fluids containing HBV (sexual intercourse) that come into contact with mucosa, lesions, or microlesions in the skin. In transmission by blood, the tiniest amounts contaminating syringe needles, ear-piercing needles, tattooing instruments, etc. suffice to produce an infection. Hepatitis B infections from blood transfusions have been greatly reduced by thorough screening of blood donors for HBs antigens, despite which patients receiving multiple transfusions or dialysis remain a high-risk group.

Another high-risk group includes all healthcare workers with regular blood contact. All blood samples must be considered potentially infectious and handled only with disposable gloves. Addicts who inject drugs with needles are also obviously exposed to a very high level of risk.

Since no effective chemotherapy against HBV has been developed to date, the WHO recommends general hepatitis B prophylaxis in the form of active immunization with HBs antigen. In response to a sudden high-level infection risk (accidental inoculation with infectious material), persons whose immune status is uncertain should also be passively immunized with human anti-HBs antiserum—if possible within hours of pathogen contact.

It has not yet proved feasible to grow HBV in vitro. The antigen used in vaccinations can be isolated from human blood. Fear of AIDS infections has resulted in emotionally based, unjustifiable rejection of this vaccine. An alternative vaccine is now available based on developments in genetic engineering: the HBs antigen can now be synthesized by a yeast fungus.

Prevention: hepatitis B booster vaccines. Periodic booster shots, especially for persons at high risk, were recommended for some time to maintain sufficient immune protection. However, since all successfully vaccinated persons build up immunity rapidly following renewed contact with the pathogen ("immunological memory," see p. 94), this recommendation has been replaced in a number of countries by the following scheme:

Following immunization on the classic model (0, 1, and 6 months), the anti-HBs antibody titer is measured within one to three months. Responders (titer 100 IU/l) require no booster. In hyporesponders and nonresponders (ti-

ter <100 IU/l), an attempt should be made to reach a titer of 100 IU/l with a maximum of three further vaccinations.

RNA viruses

Viruses with Single-Stranded RNA Genomes, Sense-Strand Orientation

To date, six viral families with single-stranded RNA genomes in sense-strand orientation are known: picornavirus, calicivirus, togavirus, coronavirus, flavivirus, and retrovirus (common orthography: picornavirus, retrovirus, etc.).

Picornaviruses

- The important human pathogenic genera of picornaviruses are:
  - **Enteroviruses** with the polioviruses (poliomyelitis), cocksackieviruses and echoviruses.
  - **Parechoviruses** types 1 and type 2.
  - **Hepatoviruses** with the hepatitis A virus.
  - **Rhinoviruses**, common cold viruses (rhinitis).

Transmission of enteroviruses, parechoviruses, and hepatoviruses is by the fecal-oral route. The viruses first replicate in the intestine, from which location they reach their target organ with the bloodstream. Large numbers of inapparent infections are typical of this group.

Rhinoviruses are transmitted by droplet infection and remain restricted to the upper respiratory mucosa.

Diagnosis: enteroviruses and parechoviruses are diagnosed by isolation in cell cultures or with PCR, hepatitis A serologically (IgM) and rhinoviruses, if at all, by isolation.

Prevention: basic polio immunization with dead or live vaccine; hepatitis A with dead vaccine; exposure prophylaxis with rhinoviruses.

The picornaviruses (Fig. 8.**12**: polioviruses) are among the most thoroughly studied viruses of all. The name *picorna* is an abbreviation that stands for two characteristics of this family: they are small (*pico*) viruses with an RNA genome (*rna*). The RNA is polyadenylated at its 3′ end and has no cap at the 5′ end, but instead a virus-coded, basic protein about 2 kDa in size, the VPg

Poliovirus

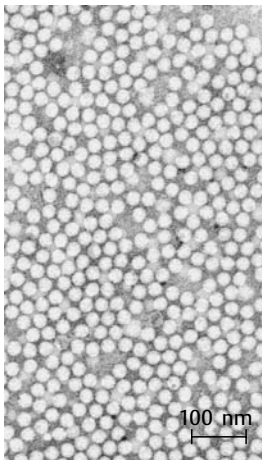

Fig. 8.**12** Polioviruses are 24–30 nm in size and cause poliomyelitis.

(**v**irus **p**rotein, **g**enome-linked). It consists of approximately 7500 bases forming 2207 coding triplets (in the poliovirus). The combination of the ribosomes with the RNA is not, as in cellular mRNA, at the cap of the 5′ end ("scanning model") but rather internally in the nontranslated region (NTR), which is about 750 nucleotides long and is positioned before the coding segment. The translation product of this RNA is a precursor polyprotein about 250 kDa in size, which is proteolytically divided into about 20 individual, functional proteins during or immediately after its synthesis. The N-terminal end of the polyprotein contains the capsid proteins, the middle region proteins that contribute to the expression of the structures required for RNA replication and formation of the CPE (p. 392f.) and the C-terminal segment includes proteins of an enzymatic character (protease for proteolytic cleavage of the primary translation product, see above, and the RNA-dependent RNA polymerase, see p. 385) as well as VPg, which functions as a primer for RNA synthesis.

The different human pathogenic genera of picornaviruses are the enteroviruses, parechoviruses, hepatoviruses, and rhinoviruses.

Enteroviruses (Poliovirus, Coxsackievirus, Echovirus) and Parechoviruses

Pathogen. The genus *Enterovirus*, isolated from the intestinal tract, includes these species:

- Poliovirus (poliomyelitis pathogen) with three serotypes.
- Coxsackievirus, group A, with 22 serotypes.

- Coxsackievirus, group B, with six serotypes.
- Echovirus with 34 serotypes.
- Enteroviruses numbers 68–71.

The genus *Parechovirus* includes the species parechovirus types 1 and 2.

Pathogenesis and clinical pictures. The enteroviruses and parechoviruses are transmitted per os and replicate at first in the lymphoid tissue of the pharyngeal space, later mainly in the intestinal wall. They then reach their "target organs" via the bloodstream (e.g., CNS, muscles, heart, liver), followed by manifest organ infection, which, however, only develops in a small percentage of cases. Most infections run an asymptomatic course. Viremia is always present, so that even asymptomatic enterovirus and parechovirus infections confer effective immunity. The cases of manifest infection frequently run atypical courses with mild clinical symptoms. The same viral type can cause different symptoms and several different viral types can cause a given clinical symptom. Recently, severe complications have been described, mainly as a sequel to hand, foot, and mouth disease (HFMD, Table 8.**2**) in Southeast Asia.

The following clinical pictures have been described for enteroviruses and parechoviruses (Table 8.**2**):

Diagnosis. The available laboratory diagnostic tools include PCR or isolation of the virus from cerebrospinal fluid, pharyngeal smear, or lavage, with the best chances of success from stool. Serodiagnosis plays only a minor role.

Epidemiology and prevention. Humans are the reservoir of the enteroviruses. Transmission is either direct (smear infection) or in food and water.

Table 8.**2** Enteroviruses and Parechoviruses: Clinical Syndromes

Virus type	Important syndromes
Polioviruses	Poliomyelitis, paralysis, aseptic meningitis, encephalitis
Coxsackie viruses A & B, echoviruses, enterovirus 68–70	Meningitis, paralysis, pharyngitis (herpangina), pneumonia, hepatitis, maculous and vesicular exanthems, including hand, foot, and mouth disease (HFMD)
Coxsackie virus B	In addition to the above: myalgia, pleurodynia (Bornholm disease), pericarditis and myocarditis, pancreatitis, diabetes
Enterovirus 71	Acute hemorrhagic conjunctivitis, HFMD
Parechovirus 1 and 2	Respiratory and gastrointestinal ("summer diarrhea") infections

Where hygienic standards are high, droplet infections also play a significant role. Special prophylactic measures to prevent infections with coxsackieviruses or echoviruses are neither practicable nor generally necessary.

Salk introduced a dead vaccine in 1954 for poliomyelitis prophylaxis (IPV, inactivated polio vaccine) consisting of three poliovirus types inactivated by formalin. Five years later, the live vaccine (OPV, oral polio vaccine according to Sabin) was introduced, which contains three live but no longer neurovirulent poliovirus strains, either singly or in combination. The WHO plan to eradicate poliomyelitis worldwide would seem feasible with this vaccine as demonstrated by its eradication in several countries including all of South America.

Polio Vaccines: Pros and Cons

The **dead or inactivated vaccine** has the advantage of a long stability period and practically foolproof application safety. The disadvantages of this vaccine form are its high cost, the requirement for three injections and weaker or at least shorter-lived protection than is provided by the attenuated form. Work is ongoing on development of enhanced (eIPV) vaccines of this type.

The advantages of the **live vaccine** are its oral application route, low price and high level of efficiency. One disadvantage is its thermolability, resulting in reduced numbers of seroconversions (more nonresponders) in tropical countries. Another difficulty is presented by the (1 in 1×10^6) cases of paralysis (vaccination-associated paralytic poliomyelitis, VAPP) resulting from a vaccination. VAPP shows a higher level of incidence than infections by the wildtype poliovirus in industrialized countries, which has led practically all these countries to return to using IPV.

Hepatoviruses (Hepatitis A Virus)

Pathogen. The hepatitis A virus differs in some characteristics from enteroviruses, to which group it was long considered to belong. Growth in cell cultures requires long adaptation. Only one serotype is known to date.

Pathogenesis and clinical picture. The clinical picture of hepatitis A, so-called epidemic or infectious hepatitis, differs in no major particulars from that of hepatitis B (p. 429). The disease nearly always takes a benign course. Only a small number of fulminant (and sometimes lethal) or chronic courses have been described. The pathogenic process at first corresponds to that of the enteroviruses, whereby hepatitis A replicates in the intestine and then, after a brief viremic episode, attacks its target organ, the liver. Disease manifestation with this pathogen, unlike most of the enteroviruses but similar to hepatitis B, involves immunological processes.

Diagnosis is based on IgM detection due to the early presence of these antibodies in patient serum, in fact so early that a lack of hepatitis A antibodies at the onset of clinical manifestations excludes hepatitis A.

Epidemiology and prevention. Transmission is by food and water or in the form of smear infections. Infection with hepatitis A shows a clear north–south gradient: it has become virtually a travelers' disease in central Europe. Imported cases frequently cause minor outbreaks in families or schools. Active immunization with an inactivated HAV vaccine is available.

Rhinoviruses

Pathogens. The genomic organization and replication system of the rhinoviruses (117 serotypes found to date) generally match those of the enteroviruses, although they differ in that they are acid-sensitive and slightly denser.

Pathogenicity and clinical picture. The rhinoviruses, the causative pathogens of the common cold, infect the mucosa of the nasopharyngeal space (nose and throat). They remain strictly localized there and do not cause generalized infections. In rare cases, mainly in children, they are known to cause bronchitis or bronchopneumonia as well. The clinical picture is often worsened by bacterial superinfection.

Diagnosis. Laboratory diagnostics are only required in special cases of rhinovirus infection. The viruses can be grown in cell cultures.

Epidemiology and prevention. Rhinoviruses are transmitted directly, for example by contaminated hands, and partly by droplet infection as well. Infective contacts between humans appear to involve mechanical inoculation (introduction into the nasopharyngeal space with fingers). Rhinoviruses occur worldwide, with pronounced proliferation in the winter months. The fact that everyone comes down with colds repeatedly is explained by the very brief immunity conferred by infection and the many different viral types involved. Experiments have shown that the infections are always exogenous, i.e., not reactivations due to cold, wetness, etc. The only conceivable prophylactic measure is to avoid large groups of people.

Astrovirus and Calicivirus; Hepatitis E

Astroviruses, measuring 28–30 nm and caliciviruses, 30–35 nm, are enteritis pathogens in small children. Human pathogens in these groups include the Norwalk virus and hepatitis E virus (HEV). The latter occurs epidemically and endemically in Asian, Central American, and African countries. It is transmitted by the fecal-oral route, above all via drinking water, and causes relatively benign infections except in pregnant women. Hepatitis E is considered a traveler's disease.

Isolated cases and minor outbreaks of enteritis are typically attributed to unspecified viral infections. Besides unidentified bacterial infections, the viral pathogens that can cause such infections include adenovirus, rotavirus, astrovirus, and calicivirus, whereby the taxonomy of the latter two have not been confirmed.

Astroviruses

Pathogen. The astrovirus is 28–30 nm in size and owes its name to its starlike appearance. It contains sense RNA with approximately 7 500 nucleotides and appears to have a replication strategy similar to that of the picornaviruses.

Pathogenesis and clinical picture. Astroviruses that are animal and human pathogens are associated with episodes of diarrhea that nearly always run a harmless course. The etiological role of these viruses has still not been clarified. Astroviruses appear to possess only a low level of pathogenicity. It should be mentioned at this point that the role of viruses in enteritis is frequently exaggerated.

Diagnosis. Detection by means of electron microscopy.

Epidemiology. Astroviruses occur worldwide. They tend to infect young children and older persons weakened by other diseases.

Caliciviruses

Pathogen. Caliciviruses are 30–35 nm, possess only one capsid protein and a polyadenylated, 7500-nucleotide RNA with a VPg at the 5′ end. The surface of the viruses has a characteristic structure with small, regular, calyxlike concavities that give the capsid the form of a Star of David.

Caliciviruses are classified based on genomic similarities as either human caliciviruses (HuCV) or "small, round-structured viruses," SRSV. This designation stems from their initial identification under the electron microscope as "small, round, virus particles." The SRSV are grouped in two subtypes, I and II. Type I includes the Norwalk virus and a number of similar viruses named for their geographic venues, some with antigenicity differing from the Norwalk type.

Clinical picture. Caliciviruses cause enteritis. Together with rotaviruses (p. 456) and adenoviruses (p. 416), they are the most frequent viral enteritis pathogens in children, often causing minor epidemics during the winter months ("winter vomiting disease").

Diagnosis. Detection by means of electron microscopy or antigen assay in stool.

Epidemiology. Two-thirds of the adult population in the temperate zone carry antibodies to the Norwalk virus. SRSV are regularly implicated in minor epidemics and family outbreaks. The transmission route of the Norwalk virus has been described: in addition to the fecal-oral route, water and uncooked foods are involved.

Hepatitis E Viruses

Pathogen. An infectious inflammation of the liver endemic to Asia, Central America, and parts of Africa is apparently transmitted by the fecal-oral route. The RNA genome of the culprit agent has now been sequenced and the virus in question, the hepatitis E virus, has been classified with the caliciviruses. It occurs in at least 13 variants divided into three groups. In-vitro culturing of HEV has not succeeded to date.

Pathogenesis and clinical picture. The clinical course of hepatitis E infections tends to be benign and resembles that of hepatitis A. It shows no chronicity. However, infections in the third trimester of pregnancy have a lethality rate of 10–40%.

Diagnosis. The antibodies can be detected by means of an enzyme immunoassay. Apparently due to cross-reactions with other caliciviruses, the specificity of the results is uncertain. A diagnosis is often arrived at based on clinical evidence and medical history (travel to endemic areas).

Epidemiology. HEV causes repeated outbreaks of considerable dimensions in the parts of the world mentioned above. The infections can be traced to contaminated drinking water. Hepatitis E is imported to central Europe as a traveler's infection, although apparently less frequently than hepatitis A. No specific prophylactic measures exist.

Togaviruses

The togavirus family (*Togaviridae*) comprises two genera. ***Alphavirus*** infections are transmitted by arthropods and are imported to central Europe mainly by travelers to tropical and subtropical countries. Their clinical pictures are variable, but almost always include joint pain (arthralgias). The most important representative of the genus ***Rubivirus*** is the rubella virus, the causative agent in German measles. This normally harmless childhood disease can cause severe embryopathies during the first trimester of pregnancy.

Pathogen. The term togaviruses formerly included a variety of viruses, including what we now classify as the flaviviruses. As defined today, the togaviruses include the zoopathic pestiviruses, one species of rubivirus, the rubella virus and the alphaviruses with 25 species. The alphaviruses most important to travelers are the Chikungunya virus (Africa, Asia), the Sindbis virus (Africa, Asia, Australia), the Ross River virus (Australia, Oceania), and the Mayaro virus (South America), which are transmitted to humans by bloodsucking mosquitoes.

Togaviruses possess an icosahedral capsid and a closefitting envelope. The capsid measures 35–40 nm and the entire virion 60–65 nm. The genome of the togaviruses is a single-stranded, polyadenylated, sense RNA. Replication not only produces new 40S genomic RNA, but a subgenomic 26S RNA fragment as well, which codes for the capsid proteins. Viral progeny are released by "budding" at the cell surface.

Pathogenesis and clinical picture. The arthropodborne alphaviruses, zoonoses of the tropical and subtropical regions, frequently cause asymptomatic or benign infections with fever, exanthem, and joint pain. Occasionally, however, persistent arthralgia and polyarthritis (lasting months or even years) do occur, sometimes involving joint destruction. Even rarer, sequelae include encephalitis and meningoencephalitis with high lethality rates.

"German measles" is a harmless exanthemous infection in children and youths, caused by a rubivirus, the rubella virus, and transmitted by direct contact. The infections remain inapparent in nearly half the cases. The virus at first replicates in lymphoid organs at the portal of entry and in the nasopharyngeal space, after which a viremia develops before the exanthem manifests. In pregnant women, the virus takes this route through the placenta to the embryo, where it can cause congenital deformities or embryonic death, especially in the first three months of pregnancy. The organs in the developmental stage in this trimester are most seriously affected by the rubella infection. The most frequent congenital deformities are deafness, cataracts, cardiac defects, microcephaly, and spina bifida. In intrauterine embryo deaths due to rubella infections the immediate cause of death is usually myocardial damage. A measles infection confirmed by IgM detection or a raised antibody count is therefore an indication for a first-trimester abortion.

Diagnosis. Serodiagnosis is the method of choice in suspected alphavirus and rubivirus infections. EIA methods are also available for IgM detection.

Prevention. There are vaccines to protect against alphavirus infections and rubella. The main aim of rubella prophylaxis is to prevent rubella-caused embryopathies. Since 10–15% of young adults are still susceptible to rubella infections and a live vaccine with few side effects that confers reliable immunity is available, serial vaccination of children (boys and girls!) is done before

puberty. The vaccine is tolerated so well that prior immune status checks are not required.

Arboviruses

The term "arbovirus" (arthropodborne virus) was originally used as a synonym for togavirus. It is now no longer an official taxon since it refers only to the arthropod vectors, whereas the variety of virus types transmitted by this route is much greater, including for instance togavirus as well as flavivirus types.

Flaviviruses

Viruses in the flavivirus family (*Flaviviridae*) include the genera *Flavivirus*, *Hepacivirus*, and *Pestivirus*. **Flaviviruses** (the prototype being the yellow fever virus [Latin: *flavus*, yellow]) are transmitted by arthropods. They cause a biphasic infection that can have serious consequences (hemorrhagic fever with a high lethality rate). In southern and eastern countries, these viruses are significant human pathogens. Only one representative of this family, the tick-borne encephalitis pathogen, is encountered in Europe.

The **hepaciviruses** (**hepatitis C** [HCV] and **hepatitis G viruses**) are not arthropodborne. HCV is transmitted mainly in blood (transfusions, blood products, intravenous drug use) and is a frequent cause of chronic disease (70% of cases), including cirrhosis of the liver and hepatocellular carcinoma. The hepatitis G virus (HGV) is related to HCV and has not been characterized in detail as yet.

Pestiviruses are only important in veterinary medicine.

Flaviviruses show morphological uniformity with an icosahedral capsid and closefitting, spiked envelope. The size of the capsid is about 30 nm and the whole virion measures 45 nm. The genome of the flaviviruses is a single-stranded sense RNA about 10 kb in size. It codes for three structural and seven nonstructural proteins. Both cotranslational and posttranslational protein processing (cleavage, p. 388), similar to what is seen in the picornaviruses, has been described. The morphogenesis of the virus occurs at the endoplasmic reticulum, into the lumen of which the finished viruses bud. These characteristics have not been directly demonstrated for the hepatitis C virus, which cannot be cultured in vitro.

The pestiviruses cause severe animal epidemics (e.g., swine fever). They are not transmitted by arthropods.

Flavivirus (Arthropodborne Yellow Fever Type)

Pathogen. The flavivirus family includes 63 species, among them the prototypic virus of the family, the yellow fever virus, and the pathogen that causes European tickborne encephalitis (spring-summer meningoencephalitis, SSME). Table 8.**3** lists the flaviviruses that cause significant travelers' diseases.

Pathogenesis and clinical picture. The arthropodborne flaviviruses cause diseases of different levels of severity. The infections are typically biphasic with an initial, not very characteristic phase including fever, headache, muscle pain, and in some cases exanthem (Denguelike disease). This phase in-

Table 8.**3** Overview of the Most Important Flaviviruses (arthropodborne)

Viral species	Transmitting vector	Geographic spread	Syndrome
Dengue	Mosquito (*Aedes*, *Stegomyia*)	West Africa, Pacific, South and Southeast Asia, Caribbean, Venezuela, Colombia, Brazil	Dengue syndrome, DHF, DSS
Yellow fever	Mosquito (*Aedes*)	West and Central Africa, South and Central America	Hemorrhagic fever
Japanese B encephalitis	Mosquito (*Culex*)	East, Southeast and South Asia, western Pacific	Encephalitis
St. Louis encephalitis	Mosquito (*Culex*)	North and Central America, Brazil, and Argentina	Encephalitis
West Nile fever	Mosquito (*Culex*), ticks (*Argasidae*)	East and West Africa, South and Southeast Asia, Mediterranean countries, recently USA as well	Dengue syndrome, encephalitis
Tickborne encephalitis (Central European* and Russian)	Ticks (*Ixodes*)	Central Europe, Russia	Encephalitis

* Syn. spring-summer meningoencephalitis (SSME)

cludes a pronounced viremia. The illness, in this stage often not recognized as a flavivirus infection, may then be over or it may progress after one to three days to a second, severe clinical picture: a hemorrhagic fever with a high lethality rate involving hemorrhages and intravasal coagulation. In Dengue fever, this form is becoming more and more frequent and is called Dengue hemorrhagic fever (DHF) or Dengue shock syndrome (DSS) depending on the predominant characteristics.

Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS)

One reason for the manifestation of more severe Dengue courses is the increasing mobility of the populace. This leads to the phenomenon of people overcoming one Dengue infection, then traveling to an area where a different Dengue serotype is endemic. What happens if they are infected again is known as an "antibody-dependent enhancement of viral infection" or ADE, see p. 399). Antibodies from the first infection attach to the viruses of the fresh infection, which are, however, not neutralized, but instead allowed entrance into cells via Fc receptors, resulting in DHF and DSS. Children still carrying antibodies from their mother during the first year of life can also experience these severe infection courses due to the same mechanism.

Diagnosis. A flavivirus infection always involves viremia (transmission by bloodsucking arthropods!). The viruses can be isolated from blood by inoculating cell cultures or newborn mice. In autopsies of fatal cases they can be isolated from liver tissue. The viruses are labile by nature and identification can take time, for which reason the diagnostic focus is on serology (titer rise or IgM detection).

Epidemiology and prevention. A cycle of infection involving a vertebrate host (mammals, birds) and a transmitting vector (bloodsucking mosquitoes and flies, ticks) has developed for most flavivirus infections. The cycles are efficient for the virus and relatively harmless for the host. The vertebrate host frequently shows few signs of disease and recovers from the infection after a brief viremia. During this period, the bloodsucking vector is infected, which thereafter remains a lifelong salivary secretor and thus infectious. In ticks, transovarian transmission of the virus is also possible. The human host is a dead end for the virus, not a normal component of the cycle. Exceptions to this are Dengue fever and urban yellow fever.

Humans are the only known main hosts for the Dengue virus. There are two forms of yellow fever: rural or jungle ("sylvatic") yellow fever with a monkey-mosquito-monkey (sometimes human) cycle and urban yellow fever with humans as the main hosts and *Aedes* mosquitoes as the transmitting vectors. This form is on the upswing due to increasing numbers of breeding places (e.g., empty tin cans in garbage piles) for the vector. Another "new" (more accurately: revived) infectious disease is the West Nile viral infection,

observed for the first time in the USA (New York) in 1999, apparently introduced into the area by migrating birds. It is still not known why the geographic distribution of the virus or infected birds changed.

Vaccines are available against yellow fever (live vaccine) and European tickborne encephalitis (dead vaccine).

Hepaciviruses (Hepatitis C and G)

Pathogen. A series of hepatitis infections following blood transfusions was observed that could not be identified as either hepatitis A (p. 437) or hepatitis B (p. 429), and were therefore designated as "non-A-non-B (NANB) hepatitis." The discovery of the hepatitis C virus (HCV) by molecular biological means in 1988 was an elegant piece of work: RNA was extracted from the plasma of an infected chimpanzee, from which cDNA was produced using reverse transcriptase. The cDNA was then cloned and the corresponding proteins expressed. About one million clones were tested for reactivity with sera from patients suffering from chronic NANB hepatitis. A protein was found by this method that reacted with antibodies to NANB, whereupon the corresponding cloned DNA was used as a probe to identify further overlapping gene segments. They belong to a flavivirus with approximately 10 kb sense RNA and several genotypes. A similar strategy led to identification of a further flavivirus that also causes hepatitis, now known as the hepatitis G virus (HGV).

Pathogenesis and clinical picture. Hepatitis C resembles hepatitis B in many respects. One major difference is that it much more frequently produces a persistent infection (85%) and, in 70% of cases, develops into a chronic hepatitis, resulting in cirrhosis of the liver within 20 years and a hepatocellular carcinoma (HCC) in a further 10 years. The reason for the high level of viral persistence is thought to be a pronounced mutability facilitating evasion of the immune defenses (quasispecies of RNA viruses, p. 391).

Diagnosis of hepatitis C is done with antibody EIA using genetically engineered viral proteins. Western blot can be used to confirm the result. The RNA can be detected by means of RT-PCR and the course of therapy can be monitored with quantitative PCR.

Epidemiology and prevention. The incidence of HCV in Europe is about 0.3%, with a decreasing tendency in the younger segment of the population. About 50% of acute hepatitis cases are HCV infections. Transmission is by blood and blood products. High-risk persons include dialysis patients, healthcare staff, and needle-sharing drug consumers. Perinatal transmission is possible, but sexual contact does not appear to be a risk factor. The transmission route is not apparent in many cases, giving rise to the expression "community-acquired infection." Feasible protective measures are the same as in hep-

atitis B; no immunization by vaccine is available. Especially in combination with ribavirin (Table 7.**5**), therapeutic use of interferon can lead to elimination of the virus in persistent infections and thus to prevention of cirrhosis of the liver and HCC.

Coronaviruses

■ Infections with coronaviruses are widespread in humans and animals. Human pathogens include causative agents of rhinitislike infections and the virus of the "severe acute respiratory syndrome" (SARS), which first erupted in China in 2002.

Diagnosis: serology or electron microscopy for common cold strains; PCR or isolation for SARS. ■

Pathogen. The *Coronaviridae* family includes several viral species that can infect vertebrates such as dogs, cats, cattle, pigs, rodents, and poultry. The name (corona, as in wreath or crown) refers to the appearance of the viruses (Fig. 8.**13**). One coronavirus species (human coronavirus, HuCV) is known since some time to be a human pathogen. It has at least two serotypes and probably a number of serological variants. In November 2002, a new coronavirus emerged in China and, after originally being mistaken as a new influenza recombinant, was identified as the causative agent of severe acute respiratory syndrome, or SARS, in spring 2003. Its origin, possibly from animals, is not known to date.

Coronavirus

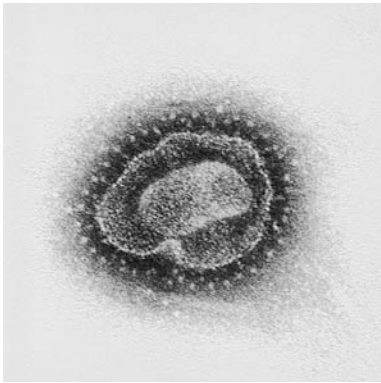

Fig. 8.**13** "Spikes" with club or drumstick-like swellings are located at regular, relatively generous intervals on the pleomorphic envelope, which measures 80–220 nm in diameter.