Symptomatology and Cytopathology

Generally, each member of the group has certain hosts in which the virus can spread systemically, causing mosaic or mottling symptoms, and other hosts in which the infection is confined to local lesions. Stunting of the host plant is sometimes observed, as is occasional systemic wilting and seed discoloration.

Infection of plant cells with comoviruses results in a number of characteristic cytological changes. These include the appearance of viral particles, either individually or as crystalline arrays, in the cytoplasm, a proliferation of cell membranes and vesicles in the cytoplasm, the appearance of amorphous inclusion bodies near or surrounding the nucleus and a variety of modifications to plasmodesmata. In the case of CPMV, the ability to cause membrane proliferation has been shown to be associated with RNA1.

Economic Importance

At present, BPMV, CPMV, CPSMV and RCMV are considered to be significant pathogens of legumes. Infection of soybeans with BPMV alone can cause yields to be reduced by 10–17%; however, this figure can rise to 60% in plants doubly infected with BPMV and the potyvirus, soybean mosaic virus. In Nigeria, infection of cowpeas with CPMV causes a great reduction in leaf area, flower production and yield. Infection of cowpeas with CPSMV has been shown to cause a 50% reduction in plant fresh weight and in the number and weight of pods. RCMV is of economic importance in forage production as it sometimes heavily invests clover. SqMV is a significant pathogen of cucurbits, problems associated with it being exacerbated by its high rate of seed transmission.

See also: **Fabaviruses (*Comoviridae*); Plant virus disease – economic aspects; Polioviruses (*Picornaviridae*): General features, Molecular biology; Virus structure: Atomic structure, Principles of virus structure; Nepoviruses (*Comoviridae*).**

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CORONAVIRUSES (*CORONAVIRIDAE*)

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Classification

The *Coronaviridae* were recognized as a new virus family in 1968 because their virion morphology and intracellular budding site distinguished them from other RNA viruses. Characteristic features of their genome, replication strategy, structural proteins and polymerase later supported this classification. The toroviruses and coronaviruses were recognized as separate genera within the *Coronaviridae* family in 1993. The *Coronaviridae* and *Arteriviridae* are now classified as members of the *Nidovirales* order, viruses with monopartite plus-strand RNA genomes that are transcribed to yield a nested set of overlapping subgenomic mRNAs that have a common 3′ end.

Virion Structure and Proteins

A model of coronavirus virions is shown in **Fig. 1.** The enveloped virions are approximately 100 nm in diameter, and are characterized by large, petal-shaped spikes. The spikes are oligomers of the 180–200 kDa S

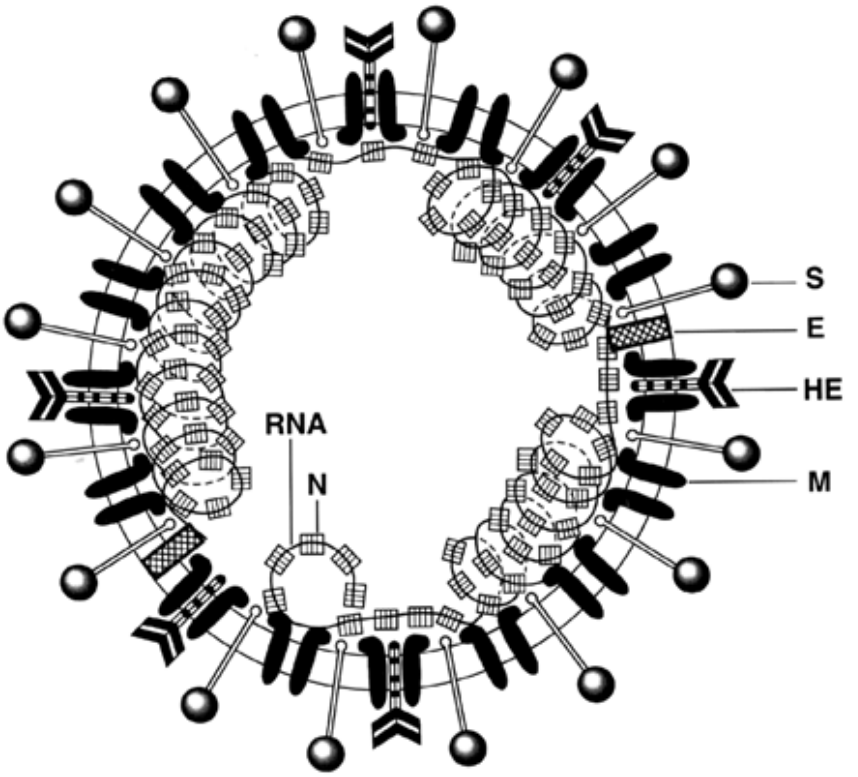

Figure 1 Model of coronavirus virion. The helical nucleocapsid containing the 30 kb, plus-sense RNA genome is coiled within an envelope that contains the S, M and E glycoproteins. Some coronaviruses related to MHV also express the HE glycoprotein. (Modified and published with permission from Fields BN, Knipe DM, Howley PM *et al* (eds) (1996) *Fields Virology*, 3rd edn. Philadelphia: Lippincott-Raven).

glycoprotein that binds to receptor glycoproteins and induces fusion of the viral envelope with cell membranes and, sometimes, cell–cell fusion. S proteins of several coronaviruses also bind to 9-*O*-acetylated sialic acid. A small envelope glycoprotein, M, traverses the lipid bilayer three times and interacts with the nucleocapsid in the virion. Intracellular transport of the M glycoprotein is arrested in the Golgi, which may determine the intracellular budding site of coronaviruses. The small E glycoprotein, originally believed to be a nonstructural protein, is present in small amounts in virions, and is essential for virus budding. Envelopes of some coronaviruses also contain a hemagglutinin-esterase glycoprotein, HE, which forms short spikes that bind *N*-acetyl-9-*O*-acetylneuraminic acid or *N*-glycolylneuraminic acid and have esterase activity. The nucleocapsid protein, N, encapsidates the monopartite, linear, single-stranded genomic RNA. The internal structure of coronaviruses was originally believed to be a helical nucleocapsid as shown in **Fig. 1**. Recently, however, cores that appear to have cubic symmetry were observed in detergent-treated virus preparations. Thus, the internal structure of coronaviruses is not yet understood.

Genome Structure

The 27–32 kb, plus-strand RNA genomes of coronaviruses are capped and polyadenylated. Transfection of genomic RNA into cells leads to production of infectious virions. The genomes of several coronaviruses have been sequenced, although no infectious coronavirus cDNA has yet been obtained. A map of the genome of murine coronavirus MHV (**Fig. 2**) illustrates the characteristic features of coronavirus genomic RNA. At the 5′ end of the genome is a cap with a leader RNA of approximately 70 bp. The order of the genes encoding the polymerase and structural proteins is the same in genomes of all coronaviruses (Pol, S, E, M, N), but several additional open reading frames (ORFs) are interspersed among these genes. These ORFs encode nonstructural (NS) proteins of unknown functions, and they differ in number, position and sequence among different coronaviruses.

For both *Coronaviridae* and *Arteriviridae*, the RNA-dependent RNA polymerase gene at the 5′ end of the genome consists of two ORFs in different reading frames, joined by a pseudoknot at a ribosomal frame shift site. The 5′ and 3′ ends of the genome contain predicted complex stem loop structures, and

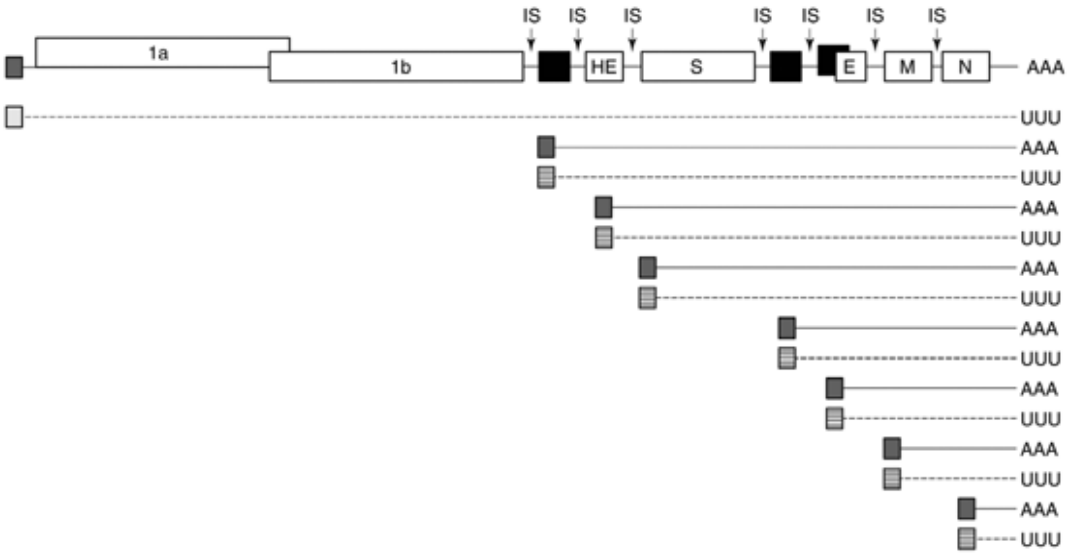

Figure 2 Map of the genome of murine coronavirus MHV and intracellular coronavirus RNAs. The polyadenylated plus-strand RNA genome is approximately 32 kb long. ORFs that encode structural proteins are shown in open boxes with the protein encoded by the gene inside. This gene order is the same in all coronaviruses. The RNA polymerase gene at the 5′ end of all coronavirus genomes consists of two ORFs, 1a and 1b, with a pseudoknot and a slippery sequence in the overlapping region. ORFs encoding putative nonstructural proteins (shown in filled boxes) vary in number and position among different coronaviruses. The conserved intergenic sequence on all plus-strand RNAs is shown by IS. The cap and approximately 70 bp leader RNA at the 5′ ends of each plus-strand RNA are indicated by a shaded box. Minus-strand templates are shown by dashed lines with a sequence complementary to the leader at the 3′ end indicated by a barred box, and a poly(U) tract at the 5′ end. Each subgenomic mRNA is translated to yield only the protein encoded by the 5′ end of the plus-strand RNA.

additional stem loops are predicted within some ORFs. Preceding every ORF in the genome is an intergenic sequence (IS) consisting of one or more UCUAAAC sequences for MHV or related sequences for other coronaviruses. The IS is included in a promoter that regulates the transcription of different viral genes. The location on the genome of packaging signals for the initiation of encapsidation of genomic RNA by the N protein appears to differ among coronaviruses.

Transcription, Replication, Translation and Genetics

Upon infection, the two large ORFs in the 20 kb polymerase gene of the genomic RNA are translated, via ribosomal frameshift, as a single, very large polyprotein that is cleaved during translation by 3-C-like and papain-like proteases within the nascent protein to yield a variety of peptides. The processing of the large polyprotein and the functions of the resulting peptides in RNA-dependent RNA polymerase activity are being analyzed in many laboratories.

Using the genomic RNA as template, the polymerase generates a full-length, minus-strand RNA with a 5′ poly(U) sequence. Infected cells also contain a 3′ coterminal nested set of polyadenylated subgenomic plus-strand mRNAs that have a cap and the approximately 70 bp leader sequence at their 5′ ends (**Fig. 2**). A negative-strand RNA template corresponding to each subgenomic mRNA is also found in infected cells (**Fig. 2**). A nonprocessive, leader-primed polymerase activity was postulated to account for the presence of the leader on each mRNA. A complex of polymerase and leader transcribed from the 3′ end of the full-length negative-strand template would bind to an IS on the template and act as a primer for synthesis of the subgenomic mRNA. This hypothesis does not explain the presence of the negative-strand templates for each subgenomic RNA. An alternative hypothesis suggests that polymerase jumping occurs during negative-strand synthesis, generating the subgenomic negative-strand templates for subsequent mRNA synthesis. Mutations in the IS preceding an ORF can prevent transcription of the mRNA with that ORF at its 5′ end. Although virus strains differ in the relative amounts of various mRNAs in infected cells, there is little temporal regulation of transcription.

Coronavirus replication is associated with a high frequency of mutations that arise by two mechanisms. First, during the replication of each approximately 30 kb RNA genome, several point mutations would

be expected to occur, based upon the known error frequency of RNA polymerases. Second, coronavirus genomes undergo a very high frequency of RNA recombination. Small or large deletions at the sites of recombination can produce mutant viruses, or defective interfering (DI) RNAs and subgenomic replicons. Large deletions in certain sites of the gene encoding the S glycoprotein and mutations in the N gene yield viruses with altered virulence, tissue tropism, or thermal stability. Thus, even after sequential single plaque isolations, coronavirus stocks always contain a mixture of quasispecies. Different virus variants may be selected during passage in different cell lines or under a variety of culture conditions. Therefore it is important to document the passage history of coronavirus strains or mutants. Rarely, recombination may occur between coronavirus genomic RNA and mRNAs of cells or unrelated viruses. Such an illegitimate RNA recombination event may have resulted in the incorporation of the mRNA that encodes the HE glycoprotein of influenza C into the genome of a coronavirus ancestral to the MHV group. Similarly, some of the NS ORFs may have been acquired through recombination with foreign mRNAs.

Genetic analysis of coronaviruses is somewhat limited because no full-length, infectious cDNA clones of coronavirus genomes are yet available. DI RNAs transfected into cells infected with wild-type virus can be replicated and have been used to study encapsidation and transcription initiation signals. Site-specific mutations have been introduced into several coronavirus genomes by targeted RNA recombination in infected cells transfected with a mutagenized subgenomic cDNA.

Coronavirus replication occurs in the cytoplasm and does not require the nucleus. Only the ORF at the 5′ end of each of the coronavirus mRNAs is translated within the infected cell or *in vitro*. The phosphorylated N protein, which is translated from one of the smallest mRNAs, is the most abundant viral protein in infected cells. It assembles with genomic plus-strand RNA in the cytoplasm to form helical nucleocapsids. Viral glycoproteins are translated on the rough endoplasmic reticulum, where S oligomerizes, then they are transported to the Golgi, where S proteins of some coronaviruses are cleaved by a trypsin-like host cell protease to yield S1 and S2 peptides. The S and HE glycoproteins are expressed on the plasma membrane. The viral S, HE, M and E glycoproteins and the nucleocapsid assemble by budding at a special pre-Golgi compartment, and the virions are apparently transported to the cell surface in large vesicles that fuse with the plasma membrane to release virions from the intact cell by exocytosis. Infected cells are characteristically coated with a thick layer of adsorbed virions.

Host Range, Tissue Tropism and Virus Propagation

Most coronaviruses cause epidemic disease in only one species, although limited replication, usually without disease, may result from experimental inoculation of other species. Coronaviruses typically cause respiratory or enteric diseases, although several can also cause hepatitis, infectious peritonitis, nephritis, myocarditis, sialodacryadenitis, or neurological, reproductive or immunological disorders. The viruses were named for their natural host and sometimes for the associated disease: for example, avian infectious bronchitis virus (IBV); mouse hepatitis virus (MHV); sialodacryadenitis virus of rats (SDAV); bovine coronavirus (BCoV); porcine hemagglutinating encephalomyelitis virus (HEV); turkey bluecomb coronavirus (TCoV); human respiratory coronaviruses (HCoV); transmissible gastroenteritis virus of swine (TGEV); porcine respiratory coronavirus (PRCV); canine coronavirus (CCoV); feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FeCoV); and rabbit coronavirus (RbCoV).

In vivo, coronaviruses bind to receptors expressed on the apical membranes of enteric and respiratory epithelial cells, and are released either from the apical or basolateral borders or both, depending upon the virus. Although strains of many coronaviruses have been adapted to growth in continuous cell lines, isolation of human coronaviruses from infected patients may require human fetal tracheal organ cultures. Some coronaviruses, such as rabbit coronavirus or enterotropic strains of MHV, cannot be propagated in cell culture, but require animal passage. Coronavirus-like particles seen in electron micrographs of human feces (human enteric coronaviruses, HECV) have not been adapted to serial passage in cell culture. IBV and TCoV can be propagated in embryonated eggs, and some strains grow in avian cell lines. Although cells of the natural host species are generally required for infection by virions, purified coronavirus genomic RNA can infect cells across species barriers. Receptors have been identified for several coronaviruses: MHV uses murine biliary glycoproteins in the immunoglobulin superfamily; HCoV-229E and TGEV use human and porcine aminopeptidase N (APN), respectively; FIPV and FeCoV use feline APN, which can also be utilized by HCoV-229E and TGEV; and BCoV and HCoV-OC43 use *N*-acetyl-9-*O*-acetyl neuraminic acid moieties. Expression of the cloned receptor glycoproteins

in cells of a foreign species can render them susceptible to infection with coronavirus virions. Thus, coronavirus–receptor interactions are an important determinant of the species specificity of coronavirus infection.

Coronavirus infection of cells may be inapparent or cause cell fusion, vacuolization, rounding and/or cell death. Cytopathic effects are minimized, and virus yield and stability are increased at acid pH. Some strains of MHV infect cells by receptor-dependent fusion of the viral envelope with the plasma membrane, but other strains of MHV and other coronaviruses appear to enter via fusion with endosomal membranes. For some coronaviruses, cleavage near the middle of the S glycoprotein at a sequence of basic amino acids yields the noncovalently linked S_1 and S_2 peptides and enhances viral infectivity and/or cell fusion. S glycoproteins of many other coronaviruses, such as FIPV, lack the protease target sequence and do not require protease activation for infectivity or cell fusion.

Serologic and Evolutionary Relationships

There are three coronavirus serogroups. One group includes HCoV-229E, TGEV, PRCoV, CCoV, FCoV, and others. A second group includes MHV, BCoV, SDAV, HEV, HCoV-OC43 and others. Avian IBV strains make up the third serogroup. Phylogenetic analysis of coronavirus N and S genes correlate well with the division of coronaviruses into these three groups. Nucleic acid sequence analysis also shows that certain pairs of coronaviruses that cause different syndromes in the same host should probably be considered strains of a single virus. Thus, PRCoV and TGEV of swine, which cause epizootic respiratory and enteric disease, respectively, are highly homologous, except for a deletion of more than 675 nucleotides in the S gene of PRCoV. The feline coronavirus FeCoV causes epizootic enteric disease in cats, and mutant FIPV viruses that arise within FeCoV-infected animals cause fatal systemic disease.

The HE glycoprotein is encoded by a gene found in the MHV group of coronaviruses, but not in the HCoV-229E or IBV groups. Phylogenetic analysis of coronavirus HE gene suggests that BCoV and HCoV-OC43 are more closely related to each other than to MHV. The organization of the polymerase gene with two large slightly overlapping ORFs in different reading frames joined by a pseudoknot is conserved among all coronaviruses and shared by toroviruses such as Berne virus (BEV) and *Arteriviridae*. In addition, BEV encodes a protein with significant homology to HE of coronaviruses and influenza C virus.

Recombination between genomes of related coronaviruses can occur in experimentally inoculated cell cultures or animals, and also during natural outbreaks of disease in the natural host. Recombinants between IBV strains have been isolated from infected birds. Nucleotide sequencing shows that one biotype of feline coronavirus that causes epizootic disease is a recombinant between canine coronavirus and the other biotype of feline coronavirus. Mutants of MHV derived from persistently infected murine cell lines have acquired the ability to infect nonmurine cells. These observations indicate that there may be naturally occurring interactions between the genomes of coronaviruses from different species.

Epidemiology

Most coronaviruses cause epidemic or epizootic disease in only one species. Because of the great antigenic variability among coronavirus strains and because many coronaviruses replicate only in epithelia where protective immunity is relatively short-lived, reinfection is common. Infection is often inapparent, and virus particles or antigens and subsequent seroconversion are observed in healthy individuals. The viruses are enzootic or endemic in their host species, causing sporadic disease and seasonal outbreaks when enough susceptibles are available. Adults with acute, self-limited, inapparent infection transmit virus to neonates that develop clinical disease. Coronavirus diseases are more severe in neonatal animals than adults, either because preexisting immunity to related virus strains moderates infections in adults, or because the immature immune system permits higher levels of virus replication. In immunocompromised hosts, infection may be inapparent but virus shedding may be prolonged. Such individuals serve as reservoirs of virus. For example, enterotropic MHV strains are endemic in colonies of laboratory mice, sustained both by persistent infection of immunocompromised nude mice and by the continuous availability of new susceptibles due to birth or importation.

Outbreaks of coronavirus diseases are often seasonal. In humans, coronaviruses cause 15–30% of colds, predominantly during the winter months, and outbreaks of different human coronaviruses alternate at 2–3 year intervals. Outbreaks of BCoV-induced dysentery in cattle also occur in the winter. Severe enteritis due to TGEV or BCoV infection of suckling pigs or calves, respectively, occurs seasonally in correlation with breeding cycles. Outbreaks of IBV-induced respiratory disease in chickens can occur at

any time, but are most common during the winter. Stress can exacerbate coronavirus-induced diseases. Mice with inapparent MHV infection can develop hepatitis if they are subjected to immunosuppression, thymectomy, transplanted tumors or infection with unrelated organisms. Cattle with inapparent BCoV infection may develop respiratory disease during shipping.

Coronaviruses have broad geographic distribution. Human infections with viruses related to HCoV-229E or to HCoV-OC43 occur worldwide, as do IBV, TGEV, BCoV, FCoV and CCoV infections. Occasionally, strains of these viruses that cause unusual manifestations of disease arise and spread locally, and some of these viruses become very widely distributed. For example, IBV strains that cause severe nephropathy arise and spread locally. PRCoV, which causes a mild epizootic respiratory infection in swine, has arisen several times in Europe and in the USA from TGEV by means of a very large deletion in the S glycoprotein. The epizootic spread of PRCoV in European pigs has apparently acted as a natural vaccine that has decreased the incidence of serious TGEV-induced enteric disease in piglets.

Pathology

Most coronaviruses cause only respiratory or enteric disease in one host species. These viruses generally replicate only in respiratory or enteric epithelial cells, where the apical membranes express specific glycoprotein receptors for the viruses. Coronaviruses are shed in respiratory secretions and/or feces. Some coronaviruses, such as some MHV strains, FIPV and rabbit coronavirus, cause disseminated disease and can replicate in macrophages, hepatocytes, neurons, glial cells, endothelial cells, kidney epithelium, lymphocytes, urogenital tract and/or myocardium. In general, coronavirus titers in the respiratory or enteric tract rise during the first 3–5 days postinoculation, and recovery of infectious virus from an immunocompetent host is usually not possible after 10–14 days, although viral antigens and RNA may continue to be detectable for several weeks. Infectious coronavirus can be shed for months by immunocompromised hosts. Although most coronaviruses do not persist in immunocompetent hosts, coronaviruses such as the neurotropic MHV-JHM strain can sometimes be detected in the brain months or years after inoculation. Reverse transcriptase–polymerase chain reaction (RT-PCR) can detect HCoV-229E and/or HCoV-OC43 RNA in up to 40% of brains from patients with neurological diseases and from healthy individuals, but the significance of this observation for human disease is unknown.

Coronavirus-induced lesions vary markedly depending upon the virus strain, dose and tissue tropism and the genetic background of the host. Intestinal infections with BCoV, MHV, TGEV, FeCoV, TCoV and CCoV cause loss of apical epithelial cells of the intestinal villi and shortening and broadening of the villi. Some enterotropic coronaviruses cause necrotizing enterocolitis, particularly in young animals, while others cause only watery diarrhea or inapparent enteric infection. Diarrhea is probably due to altered transport of fluids and electrolytes by the immature epithelial cells that cover the blunted villi. Intestinal absorption of certain sugars in TGEV-infected pigs remains altered for several days after the diarrhea has ceased. Mononuclear inflammatory cells infiltrate the lamina propria. Reinfection with a different strain of the virus generally causes more moderate disease than primary infection. However, kittens are more likely to develop FIP following their second infection with feline coronavirus, than after their first infection. Thus, an immunological response to the primary infection may somehow facilitate the later development of disseminated disease.

Human respiratory coronaviruses related to HCoV-229E or HCoV-OC43 infect the epithelial cells in the upper respiratory tract and cause colds. Infection of young asthmatic children can exacerbate wheezing, and lower respiratory tract coronavirus infection has occasionally been observed in adults. Reinfection is frequent, even in volunteers inoculated with the same strain of human coronavirus. Infection is usually demonstrated by RT-PCR or by rising serum antibody titers because primary isolation of these viruses from respiratory washings is difficult. It is not yet clear how many coronavirus strains cause human respiratory disease or whether coronaviruses may play a role in other human diseases.

IBV-induced respiratory diseases in chickens is of great economic importance. In addition, some IBV strains are nephrotropic and cause kidney disease, while others infect the oviduct and reduce egg laying. Recombinants between several IBV strains have been isolated from infected flocks.

Neurological disease and hepatitis can result from coronavirus infections. MHV strains cause local and/or systemic infections via respiratory or fecal/oral routes. While some MHV strains are strictly enterotropic, most strains infect both the respiratory and enteric tracts. Some MHV strains causes focal hepatitis, acute encephalitis, and subacute or chronic focal demyelinating disease in the murine brain and spinal cord. Cell fusion, necrosis and infiltration with mononuclear cells are observed in acutely infected tissues, depending upon the strain of virus and strain of mice. Susceptibility to MHV is affected by at least

three murine genes that determine the virus receptor isoforms expressed, the yield of infectious virus and the ability to generate monocyte procoagulant activity, a prothrombinase in the coagulation pathway, in response to MHV infection. Mutations in the MHV and TGEV S glycoproteins can alter tissue tropism, virulence and persistence. HEV causes respiratory infection and outbreaks of encephalomyelitis in suckling pigs, and virus infection of neurons that innervate the stomach causes vomiting and wasting syndrome.

Unusual syndromes associated with coronaviruses include feline infectious peritonitis, rabbit myocarditis and rat sialodacryadenitis. FECV causes enteritis in kittens and inapparent infection in adult cats, while the closely related FIPV can cause infectious peritonitis, with ascites, wasting and death in a small percentage of infected cats. A cardiotropic rabbit coronavirus causes dilated myocardiopathy and death within 7–12 days after intravenous inoculation of rabbits with serum from an infected rabbit. Rat coronaviruses infect the respiratory tract, salivary and lacrimal glands, causing sialodacryadenitis, and can also infect the urogenital tract, interfering with breeding.

Immune Responses

Infection of respiratory or enteric tracts of adults results in antiviral antibodies in the serum, secretory antibody in the respiratory and enteric tracts, colostrum and milk, and development of virus-specific T cells. These immune responses are useful for diagnostic purposes, terminate infection, and probably ameliorate subsequent infections, but they do not necessarily prevent reinfection. In newborns, passive oral immunization with neutralizing antibody can sometimes prevent fatal coronavirus enteritis. Because coronavirus diseases are generally most severe in newborns that do not respond well to active immunization, an attractive strategy to protect these newborn animals that is being explored is to vaccinate pregnant dams in order to maximize antiviral antibodies in the colostrum and milk.

Coronaviruses can sometimes infect cells of the immune system and may modulate cytokines and immune responses to unrelated immunogens. Infected macrophages can spread infection to distant tissues. Mononuclear cell infiltrates in infected tissues consist of macrophages, plasma cells, and CD4+ and CD8+ lymphocytes. Infection of glial cells with MHV can upregulate expression of major histocompatibility complex (MHC) class I or II antigens, making these cells potential targets for cytotoxic T cells. MHV infection causes thymic atrophy, and the virus can also infect B lymphocytes. Polyclonal B cell activation and hypergammaglobulinemia are associated with FIPV infection. Cheetahs, which have little polymorphism in their MHC genes, have a much higher fatality rate after feline coronavirus infection than cats.

Prevention and Control of Coronavirus Diseases

Because of the economic importance of coronavirus diseases of domestic animals, modified live vaccines against IBV, TGEV, BCV, CCV and FIPV have been developed. However, they do not provide solid protection from infection with wild-type coronaviruses. Other approaches to protection or control of coronavirus diseases include use of recombinant S proteins, synthetic peptides that mimic neutralization epitopes, passive immunization with antibody against S glycoproteins, and treatment with interferon α or monoclonal antireceptor antibody. Improvement of vaccines will require understanding of coronavirus virulence factors, strain variation and mechanisms of immunopathology.

Future Perspectives

Full-length cDNA copies of coronavirus genomes and a way to express them to obtain infectious virions are needed to investigate coronavirus replication and pathogenesis. Analysis of the complex synthesis, processing and functions of the coronavirus polymerase peptides will provide unique insight into mechanisms of RNA recombination, tools for coronavirus genetics, and possibly new targets for drugs to inhibit coronavirus replication. Improvements in diagnostic tests to identify coronavirus infections in humans and animals will elucidate the epidemiology of these viruses and may implicate coronaviruses in the etiology of additional diseases. The characterization of coronavirus–host interactions during persistent infection *in vitro* and *in vivo* will provide additional insight into coronavirus epidemiology and pathogenesis. Further understanding of virus variants and host responses to coronavirus infections of the respiratory and enteric tracts may lead to improved coronavirus vaccines.

See also: **Arteriviruses (*Arteriviridae*); Toroviruses (*Coronaviridae*); Immune response: Cell mediated immune response, General features; Respiratory viruses; Recombination of viruses; Rhinoviruses (*Picornaviridae*).**

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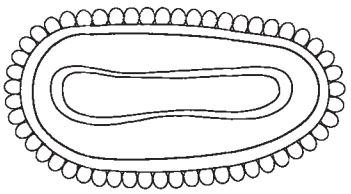
COWPOX VIRUS (*POXVIRIDAE*)



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History

The first published account of human and bovine cowpox is Edward Jenner's 'Inquiry' of 1798. He described the clinical signs of cowpox in both species, and how infection in humans with *Variolae vaccinae* ('known by the name of the cowpox') provided protection against smallpox. At that time smallpox killed 200 000–600 000 people each year in Europe and caused up to 30% of all deaths in city children. Jenner's observations, despite the concern of some over the consequences of inoculating bovine material into humans, soon led to the use of smallpox vaccine around the world. However, not until Pasteur's work nearly 100 years later was the principle of immunization used again, and it was Pasteur who suggested that all such immunizations be called 'vaccines' to honour Jenner.

Although Jenner's first vaccines came from cattle, later material was sometimes derived from horses, and the origins of modern vaccinia virus (smallpox vaccine), are unknown. Differences between cowpox and vaccinia viruses were first reported in 1939, since when further biological and genetic studies have confirmed that vaccinia virus is a separate species and not simply a mutant of cowpox virus or a recombinant of smallpox and cowpox viruses.

Even Jenner had difficulty finding cowpox, and cowpox virus is certainly not now endemic in cattle. It is now accepted that cowpox virus is endemic in rodents, with cattle and humans acting as accidental hosts. However since the 1970s, the domestic cat has been the animal most frequently seen with cowpox.

Taxonomy and Classification

Cowpox virus is a member of the genus *Orthopoxvirus* in the subfamily *Chordopoxvirinae* of the *Poxviridae* family; the international reference strain, Brighton, was isolated in 1937 from farm workers in contact with cattle. Cowpox virus can be differentiated from other orthopoxviruses by a combination of biological tests, including the ability to produce hemorrhagic pocks on chorioallantoic membranes, the production of A-type inclusions (ATI) in infected cells, and its 'ceiling temperature' (40°C, the highest temperature at which it will replicate), by minor antigenic differences, and by a variety of molecular assays including restriction enzyme digestion, particularly with Hind III, and sequencing or restriction endonuclease digestion of polymerase chain reaction (PCR) products from, for example, the ATI and fusion protein genes.

Not all strains of cowpox virus are identical and some, isolated from unusual hosts, have been referred to in the past as 'cowpox-like' viruses. However, they are clearly strains of cowpox virus, and probably represent geographic or host-range variants.

Properties of the Virion

Cowpox virus has a typical orthopoxvirus morphology, and is indistinguishable from vaccinia virus by electron microscopy. Virions are brick shaped, approximately 300 × 200 × 200 nm in size, and may be enveloped. The virion consists of a biconcave core, and within each concavity lies a lateral body (**Fig. 1**).