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CHAPTER • 18

Coronavirus Infections

EQUINE CORONAVIRUS

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Coronaviruses have been identified in a wide range of animal species as causes of a variety of primarily gastrointestinal and respiratory diseases. Although coronavirus-induced enteritis has been suspected in foals with diarrhea, direct pathogenicity of equine coronavirus (ECV) in equids has not been demonstrated.[1,2]

Etiology

Coronaviruses are members of the *Coronaviridae* family, order Nidovirales, all of which are positive-sense ribonucleic acid (RNA) viruses.[3-6] The family *Coronaviridae* contains two genera, *Torovirus* and *Coronavirus*.[3,4,6-8] The coronaviruses were so named because the unusually large, club-shaped peplomers projecting from the envelope give the viral particle the appearance of a solar corona.[4,6,9] The tubular nucleocapsid is composed of a phosphorylated nucleoprotein and seems to be connected directly to a transmembrane protein, *M*, which spans the lipid bilayer three times and has only a small fraction of its mass exposed to the external environment.[4,6,8,9] Two types of prominent spikes line the outside of the virion. The long spikes, which consist of the *S* (spike) glycoprotein, are present on all coronaviruses and give them their characteristic "corona" appearance. The short spikes, which consist of the *HE* (hemagglutinin-esterase) glycoprotein, are present in only some coronaviruses.[4,6-8] Based on antigenic relationships and genetic homologies, the coronaviruses are subdivided into three antigenic groups.[1-3,7,10] ECV is a member of the group 2 mammalian coronaviruses.[7]

Toroviruses are established agents of gastroenteritis in animals, and the type species of the genus is Berne virus (BEV), a chance isolate from a diarrheic horse in 1972.[5,8,11-13] Torovirus is discussed in more detail later in this chapter.

Epidemiology and Clinical Findings

Coronaviruses are a common cause of disease in humans and domestic animals.[6] Coronaviruses have been identified in mice, rats, chickens, turkeys, swine, dogs, cats, rabbits, horses, cattle, and humans.[1,4] They cause respiratory, gastrointestinal, neurologic, and generalized infections.[8] In horses, it is believed that coronavirus infection spreads through fecal-oral transmission; however, other routes of transmission, such as respiratory and mechanical, may also be possible.[8,14] Most coronaviruses infect only the cells of their natural host species and a few closely related species.[6] In their natural host species, coronaviruses exhibit marked tissue tropism. Virus replication in vivo can be either disseminated, causing systemic infections, or restricted to a few cell types, often the epithelial cells of the respiratory or enteric tracts and macrophages, causing localized infections. Coronavirus replication takes place in the cytoplasm of infected cells.[6]

Anzai et al.[14] investigated the effect of long-distance transport of 29 racehorses (age 2 years) on serologic evidence of infection with potential respiratory pathogens, including coronavirus. Serum antibody titers to coronavirus were evaluated by serum neutralization (SN) test using bovine coronavirus (BCV), which is closely related antigenically to ECV.[7,14,15] Two horses were seropositive for BCV 1 month before transportation (dilution titers 1:10 and 1:40). These horses were transported in the same vehicle as four horses that were seronegative to coronavirus. The four seronegative horses seroconverted after transportation (titers between 1:10 and 1:20 within 1 month), but none developed clinical signs, and a direct relationship between disease and coronavirus infection could not be confirmed.[14] This study suggests that ECV may spread among horses while they are stabled together or during transport. This conclusion is consistent with serologic evidence that BCV or its related virus is widely prevalent in horses in Japan.[14,15]

Coronavirus-like particles have been observed by negative-contrast electron microscopy (EM) in fecal samples from healthy and diarrheic foals,[16-21] from one foal with combined immunodeficiency syndrome and diarrhea,[22] and from adult horses with Potomac horse fever.[23] Concurrent infections with rotavirus[18,19] and *Cryptosporidium*[22] have also been reported.

Davis et al.[2] identified a coronavirus antigenically related to BCV in a 5-day-old foal with enterocolitis. Bacterial cultures from feces were negative for enteric pathogens, and viral particles were not observed on EM. The coronavirus was identified in intestinal tissues of the foal by immunohistochemistry using BCV-specific monoclonal antibodies and in feces using an antigen-capture enzyme-linked immunosorbent assay (ELISA) designed for BCV detection.[2,7] The foal's serum antibody titer to BCV increased over an 8-day period from 1:25 to 1:100.[2]

Despite the reports of probable or possible coronavirus infection in foals, there were no definitive descriptions of ECV isolation from sick horses before 2000.[2,16,17,22,23] In 2000, Guy et al.[7] described for the first time the isolation and characterization of ECV (isolate NC99) from feces of a 2-week-old diarrheic foal. Further study of this isolate may yield important information about the role of enteric coronaviruses in equine intestinal disease.

Diagnosis

Coronavirus infection may be suspected if other etiologic agents of diarrhea in foals have been ruled out. Coronaviruses are difficult to isolate and propagate in cell culture. The diagnostic method of choice is direct demonstration of coronavirus antigens in biologic samples.[9] Negative-stain EM can be used to identify coronavirus-like particles in feces.[7,17-19,22,23] However, if viral particles are not present in sufficient numbers, EM examination may require considerable searching or may be unrewarding.[2,16]

Because of the cross-reactivity between BCV and ECV, detection of neutralizing antibody to BCV in horses provides presumptive evidence of exposure to ECV.[2,7,14,15,17] Because the presence of SN antibodies against BCV in equine sera may be a common finding, acute and convalescent samples

should be examined for evidence of increasing titer.[2,14,15] Convalescent serum samples from horses with suspected ECV infection may be evaluated approximately 10 days after the onset of disease. In human patients, a fourfold increase in titer to coronavirus is indicative of recent active infection. An antemortem diagnostic panel for ECV should include assay for serum antibody titer to BCV and fecal capture ELISA evaluation for coronavirus antigen.[2]

Although coronaviruses or coronavirus-like particles have been identified in foals and adult horses with enteric disease, the pathogenicity of coronaviruses and their etiologic role in equine enteric disease remains unclear, and it is unlikely that coronavirus infection is responsible for outbreaks of diarrhea.[2,7,22,24] Additional studies are needed to determine the prevalence of ECV infection in healthy and sick horses, the occurrence of mixed infections of coronavirus and other enteric pathogens, and the relative importance of ECV as a cause of enteric disease in horses.[7]

EQUINE TOROVIRUS

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Etiology

Equine torovirus (Berne virus) was originally isolated from a rectal swab of a horse with hepatic and gastrointestinal disease in Berne, Switzerland, in 1972.[25] It is currently classified in the *Torovirus* genus with bovine, human, and porcine toroviruses, within the family *Coronaviridae* and order Nidovirales.[26-28] The enveloped virions are pleomorphic with large protein spikes on the surface, resembling the peplomers of coronaviruses. The nucleocapsid has a tubular appearance with helical symmetry. The positive-sense RNA genome is estimated to be 20 to 25 kilobases in length with six open reading frames (ORFs). Four structural proteins have been identified: spike (S), membrane (M), hemagglutinin-esterase (HE), and nucleocapsid (N) proteins.

Epidemiology

Although originally isolated from a horse with gastrointestinal disease, a causal link between Berne virus and equine disease has not been established. Limited seroepidemiologic studies indicate that the virus is present in Europe and the United States. Neutralizing antibody is also found in the sera of other ungulates (cattle, sheep, goats, pigs), laboratory rabbits, and at least two species of wild mice (*Clethrionomys glareolus* and *Apodemus sylvaticus*).[29]

Clinical Findings

Despite widespread evidence of exposure to Berne virus, no evidence indicates that this virus is associated with clinical disease in horses or any other species. Inoculation of the virus into two foals induced neutralizing antibody without associated clinical signs.[29] Bovine torovirus has been associated with gastroenteritis in calves and possibly pneumonia in older cattle.[28,30] Human and porcine toroviruses are associated with gastroenteritis in people and pigs, respectively.[31-33]

REFERENCES

See the CD-ROM for a list of references linked to the abstract in PubMed.

CHAPTER • 19

Rabies

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Rabies virus (RABV) is an enveloped ribonucleic acid (RNA) rhabdovirus that induces lethal polioencephalomyelitis and ganglionitis in infected animals.[1,2] The disease is universally endemic in mammals and other warm-blooded vertebrates, except in Australia, where other types of zoonotic lyssaviruses transmitted by flying foxes (bats) are present. The disease has been excluded or eradicated from some countries, or parts of them, especially islands (e.g., Great Britain, New Zealand, Iceland).

ETIOLOGY

Viruses in the *Rhabdoviridae* family known to infect mammals belong to either the genus *Vesiculovirus* (vesicular stomatitis virus serotype New Jersey and serotype Indiana, Chandipura virus, and Piry virus) or the genus *Lyssavirus* (rabies virus and rabieslike viruses).[1,2] Lyssaviruses include six distinct genotypes that can be classified according to their degree of amino acid homology. Genotypes 2 (Lagos bat virus) and 3 (Mokola virus) are the most phylogenetically distant from the vaccinal and classic rabies viruses of genotype 1. Genotypes 4 (Duvenhage virus) and 5 (European bat lyssavirus 1 [EBL1]) are closely related to each other, with the separate genotype 6 represented by EBL2.

RABV virions are enveloped, bullet-shaped, 45 to 100 nm in diameter, and 100 to 430 nm long (Fig. 19-1). Surface projections of the envelope are distinct spikes, dispersed evenly over the whole surface (except for the quasiplanar end of bullet-shaped viruses). The uncoiled nucleocapsid is filamentous, with regular surface structure, and cross-banded. Virions contain 1% to 2% nucleic acid composed of one molecule of linear, usually negative-sense, single-stranded RNA. Nucleotide sequences of the 3′ terminus are inverted and complementary to similar regions on the 5′ end and are the same for each gene segment in species of the same genus. Virions contain 65% to 75% protein, most of which are structural. RABVs are recognized and classified through panels of monoclonal