Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 27

Neonatal Calf Diarrhea Virus

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VIRUS PROPERTIES

Calf diarrhea coronavirus particles in negatively stained preparations have a mean diameter of 120 nm and are covered by petal-shaped projections about 20 nm long. Viral replication occurs in the cytoplasm. The virus is sensitive to ether, chloroform, deoxycholate and exposure to 50°C for 1 h. Thermosensitivity is stabilized in the presence of 1 *M* $MgCl_2$. The virus is stable at pH 3. Hemadsorption and hemagglutination occur with erythrocytes of hamsters, mice and rats. Formalin treatment (0.02% at 37°C for 24 h) completely inactivates the infectivity in cell culture fluid (Sharpee et al., 1976). Stability of the virus in the environment is not known. To date, one serotype has been identified.

EPIZOOTIOLOGY

Calf diarrhea coronavirus infects only the bovine species. Under natural conditions, calves 1 day to 3 or more weeks old are affected. Gnotobiotic piglets inoculated with the calf coronavirus developed no clinical signs. No recognized illness has occurred in people working with the virus. Circumstantial evidence suggests that recovered calves introduced into a healthy herd can be responsible for an outbreak of diarrhea, and that there may be carrier cows. Disease patterns and the effect of adverse conditions are the same for coronavirus as described for rotavirus infections.

Morbidity in an outbreak of diarrhea is usually high, but mortality is influenced by the age of the calf when infected, management and type of secondary infection.

PATHOGENESIS

Portal of entry is the mouth through contact with teats, feed or fomites contaminated with infected feces. Primary sites of viral replication are the mature epithelial cells on the small intestinal villi and surface epithelial cells of the colon. During the first few hours of diarrhea the feces can contain up to 10^{10} virions/ml.

DISEASE SIGNS

Uncomplicated coronavirus infection has been studied in gnotobiotic calves. The incubation period in experimental calves varies from 20 to about 36 h. In

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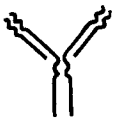
contrast to rotavirus infections, gnotobiotic calves infected with coronavirus become anorectic but do not become as depressed. The appearance of the initial diarrheic feces is the same as for rotavirus; the feces are liquid, yellow, and the volume is somewhat dependent on the amount of milk fed after inoculation. Within 12–24 h after the onset of diarrhea, coronavirus-infected calves will be hungry, but if fed milk will continue to have liquid feces which contain some curd and mucus; the volume of feces will depend on the amount of milk fed. When normal milk feedings are continued, diarrhea in gnotobiotic calves will persist for 5–6 days. Bacteria-free calves fed normal quantities of milk may die of severe dehydration 48–62 h after onset of diarrhea. If an oral electrolyte solution is fed instead of milk, the calves recover.

Although the bovine coronavirus is much more known as one of the agents of enteritis, this virus was also isolated (as a member of mixed infections) from the trachea and lungs of calves with a respiratory disease (McNulty et al., 1984). These authors showed that in newborn calves the respiratory isolates, inoculated nasally and tracheally, caused a mild respiratory disease (cough) accompanied by diarrhea. The isolates are thus probably neither genuine respiratory, nor genuine enteric strains; also it was shown by cross-serology and cross-immunization that both belong to a single serotype (McNulty et al., 1984; Reynolds et al., 1985).

PATHOLOGY

Calves necropsied during the acute or later stages of coronavirus diarrhea have no gross lesion in the small or large intestine other than an increased amount of liquid contents. Calves that die show signs of dehydration. Congestion, hemorrhage or other gross lesions, if seen in coronavirus infection are due to secondary infection.

Light microscopic, IF and EM examination of intestine from calves killed at different intervals after the onset of diarrhea revealed the following changes. At the onset of diarrhea, the small intestinal villous epithelial cells and surface colonic epithelial cells have a normal morphology by light microscopy, but are full of viral antigen and virus. Thus the initial diarrhea is believed to result from a redirection of epithelial function from absorption to virus production. As the disease progresses, the infected epithelial cells of the small intestine and colon are lost. The villi become truncated, covered by squamous to cuboidal epithelial cells, and adjacent villi particularly in the lower ileum may fuse. In the colon, the lost epithelial cells on the colonic ridges are replaced by cuboidal cells, and the ridge structure is lost. The persistence of immature epithelial cells for several days is believed to be responsible for the continued diarrhea and dehydration; the immature epithelial cells cannot complete the digestion of milk, and thus there is decreased absorption of milk and digestive fluids (Mebus et al., 1973b, 1975a).



IMMUNE REACTION

Circulating coronavirus antibody does not prevent infection (Mebus et al., 1973b), but may cause an attenuation of the infection through resecretion of antibody into the lumen of the intestine (Mebus et al., 1975b). Regular ingestion of colostrum and milk from an immune dam and the consequential presence of antibodies in intestinal lumen can apparently be protective.

Oral administration of an attenuated coronavirus to newborn calves causes no adverse reaction, and the calves are resistant to challenge inoculation when

96 h old (Mebus et al., 1975b). Isolated intestinal loops (Thiry-Vella loops) have been used to study the immune response. Virus was isolated from loop washings for 6–7 days after inoculation. Beginning at 6–7 days after inoculation, neutralizing antibody identified as IgM and IgA appeared in the loop washings, rose to a respectable titer by 10 days post-inoculation, and persisted. Circulating antibody appeared at 7–8 days post-inoculation. Thus it appears that resistance to coronavirus infection is primarily due to the presence of colostral and/or milk antibody or actively produced IgM and/or IgA in the intestinal lumen (Mebus et al., 1975b).

LABORATORY DIAGNOSIS

Coronavirus diagnosis can be made by EM examination of feces collected during the early stages of diarrhea. The specimens should be collected into a suitable container directly from an animal. When possible, specimens should be collected from several animals; specimens can be frozen. Another method of diagnosis is IF staining of frozen sections of spiral colon collected from a calf killed 1–4 days after the onset of diarrhea. Pieces of colon should be shipped frozen to the laboratory.

In fecal samples the coronaviral antigens can be detected by immunoelectroosmophoresis (Dea et al., 1979), reversed passive HA (Sato et al., 1984), and ELISA (Reynolds et al., 1985). A modified ELISA was used to detect antigen–antibody complexes in fecal samples during chronic shedding of bovine coronavirus by clinically normal cows (Crouch et al., 1985).

The bovine coronavirus has been cultivated with cytopathic effect in primary bovine embryonic kidney cells (Mebus et al., 1973a) and in a continuous line of bovine embryonic kidney (BEK-1) cells (Inaba et al., 1976). The replication of isolates could be enhanced by addition of trypsin to cells of a line of bovine embryonic lung (BEL) cells (Toth, 1982) or to cells of a line of human rectal tumor (HRT-18) (Reynolds et al., 1985). Isolates adapted to growth in HRT-18 cells could be titrated on these cells by a plaque assay (Vautherot, 1981). Bovine coronavirus isolates did replicate in organ cultures of bovine fetal trachea and intestine as was shown by EM, indirect IF and specific agglutination of rat erythrocytes (Bridger et al., 1978; McNulty et al., 1984).

PROPHYLAXIS AND CONTROL

Vaccination of cows prior to parturition will apparently increase the amount of antibody in the colostrum and milk and thus reduce the incidence and/or severity of diarrhea. However, with passive protection, the calf has to become infected to develop an active immunity. Ideally, this will be a subclinical infection.

Oral vaccination of newborn calves will induce an active immunity. However, the problem with an oral vaccine is the timing of vaccination in relation to the ingestion of colostrum and amount of antibody in the colostrum. Vaccination seems to be more successful in herds in which coronavirus diarrhea is occurring in the very young calf.

Interference by colostral antibody with an oral attenuated virus vaccine can be overcome by vaccination of the calf in utero. Attenuated coronavirus inoculated into the amnionic fluid of 7–8-month-old fetuses caused no adverse reaction, and the animals had circulating antibody when delivered by hysterotomy. These calves were not challenge inoculated, but based on the results of rotavirus challenge results of in utero vaccinated calves, one would expect them to be protected.

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Equally important to vaccination are good management practices to minimize introduction of the infection into the herd: new animals should not be introduced into the herd immediately before or during the calving season, and hygiene measures should be followed when people move between herds.

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