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Toroviruses (*Coronaviridae*)

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History

Equine torovirus (EToV), originally referred to as Berne virus, was accidentally isolated in equine kidney cells in 1972, from a rectal swab taken from a horse with diarrhea. EToV was successfully propagated in cell culture - in lines of equine dermis or embryonic mule skin cells, where it causes a cytopathic effect that results in cell lysis. While Berne virus was not neutralized by antisera against known equine viruses, serologic cross-reactions were observed in neutralization tests and ELISA using sera from calves that had been experimentally infected with morphologically similar particles, then baptized 'Breda' viruses.

The first 'Breda' bovine torovirus (BToV) was discovered in 1979 during an investigation in a dairy herd in Breda (Iowa), in which severe neonatal calf diarrhea had been a problem for three consecutive years. After this initial report, other strains of BToV were identified in beef calves from Ohio, and in a colostrum-deprived (CD) calf from Iowa. Most of the studies on the pathogenesis and pathology of torovirus infections have been done in BToV-infected gnotobiotic (Gn) and CD calves, as well as in limited field studies; in contrast, most of the biochemistry and morphogenesis data on toroviruses are based on EToV studies.

In 1984, torovirus-like (TVL) particles were detected in the feces from human patients with gastroenteritis by using electron microscopy (EM). Since then, reports of human toroviruses (HToV) in children and adults with acute diarrhea have appeared in several countries, and have been reported as a commonly identified cause of nosocomial viral gastroenteritis in neonatal intensive care units. TVL particles have also been detected in fecal samples from pigs, and named porcine torovirus (PToV), which have several lineages circulating in porcine populations worldwide. However, a link between PToV and clinical disease remains unclear.

Turkey torovirus (TToV) was detected in young poultry with stunting syndrome characterized by diarrhea, poor weight gain, and suboptimal performance. TToV has similar physicochemical properties and sequence similarity of polymerase protein gene to EToV. TToV was propagated in primary culture of turkey intestinal epithelial cells as well as in turkey embryos. Experimental inoculation of TToVs into young poult results in characteristic stunting syndrome.

In 2007, cytopathogenic BToV from a calf with diarrhea in Japan (designated as BToV-Aichi) was isolated in human rectal tumor cell line HRT-18, which is frequently used for the isolation of bovine coronavirus. Subsequently, another Japanese group isolated four more cytopathogenic BToV strains in HRT-18 cells used for the first BToV strain, but could not in HRT-18 cells used for bovine coronavirus. This indicates that cytopathogenic BToVs were preferentially propagated under the particular conditions, including the source of the HRT-18 cells used. Such adaptation is reported to be associated with the mutation of the HE gene producing a truncated hemagglutinin-esterase protein, which apparently was produced during the passage of the virus in such cultured cells.

Taxonomy and Classification

Toroviruses are single-stranded, positive-polarity RNA viruses with a peplomer-bearing envelope. The term *torus* (Latin) refers to the circular convex molding in the form of a doughnut that some columns or pilaster have at their bases; indeed it was the unique biconcave disk and C-shape of the virion in the extracellular environment that suggested this naming. Since 1992, the genus *Torovirus* has been included in the family *Coronaviridae*, based on their similar genomic organization and replication strategies. Toro- and coronaviruses are also ancestrally related: their polymerase and envelope genes diverged from those of a common predecessor. Because of their inclusion in the family *Coronaviridae*, the nomenclature for coronavirus genes, mRNAs, and structural proteins have been also applied to toroviruses. However, the lack of sequence homology in the structural genes and the absence of antigenic relatedness with coronaviruses justify their taxonomic position as a separate genus. The International Committee on Taxonomy of Viruses (ICTV) presently recognizes four species in the genus *Torovirus*: EToV, BToV, PToV, and HToV.

The families *Coronaviridae*, *Arteriviridae*, and *Roniviridae* as well as the new family *Mesoniviridae*, are the constituents of the Order *Nidovirales*, the second order in animal virology (after the order *Mononegavirales*). This assignment is based on their similar basic genomic organization and common replication strategy: the synthesis of a 3' coterminal nested set of four or more subgenomic mRNAs, and the possession of two open reading frames (ORFs) connected by a frameshift site to express a replicase directly from the genomic RNA. This nested set was the foundation for the name of the *Nidovirales* order (from Latin *nidus*, 'the nest').

Virion Properties

Toroviruses particles possess a nucleocapsid with helical symmetry coiled into a hollow tube (diameter 23 nm, average length 104 nm, periodicity 4.5 nm). Extracellular, negatively stained torovirus virions are generally observed as kidney- or C-shaped particles ($105\text{--}140 \times 12\text{--}40$ nm). They can also be seen as spherical or oval particles ($89 \pm 7 \times 75 \pm 9$ nm) or rod-shaped virions (35×170 nm), depending on the different orientations of the virions with respect to the electron beam. A graphic representation of a torovirion is shown in [Figure 1](#). A tightly fitting envelope, 11 nm thick, surrounds the virion structure bearing prominent drumstick-shaped peplomers (17–24 nm), and a fringe of shorter spikes (8–10 nm), which represent the spike and the hemagglutinin esterase proteins, respectively. Intracellularly, toroviruses are observed as elongated tubules with rounded ends (rod-shaped virions, $35\text{--}42 \times 80\text{--}105$ nm), located in the cytoplasm of infected intestinal cells.

Genome

The torovirus genome consists of a single-stranded, polyadenylated RNA of positive (messenger) polarity, which is about 28.5 kb in length. The complete genome sequence of toroviruses is reported in BToV and PToV, comprising 28 475 and 28 301 nucleotides which contain six ORFs (see [Figure 1](#)). ORF1a and ORF1b are the most proximal 5' reading frames and constitute the replicase (RNA dependent RNA polymerase) gene, which is expressed as a large precursor protein directly from the genomic RNA by a ribosomal frameshift mechanism, similar to other nidoviruses. The large product of these ORFs is apparently involved in the synthesis of a negative-strand RNA and the onset of subgenomic RNA synthesis. The other four ORFs correspond to structural protein genes and are expressed by the production of a 3'-coterminal nested set of four mRNAs. The ORF2, ORF3, ORF4, and ORF5 of BToV and PToV encode the spike (S), membrane (M), hemagglutinin-esterase (HE), and nucleocapsid (N) proteins, respectively. The bioinformatics analysis reveals CUG-initiated coding sequence, overlapping 5' end of BToV and EToV ORF1a and encoding a novel 30 kDa product. Further studies should identify a novel 30 kDa product by means of immunoassays with its specific antibodies and its function.

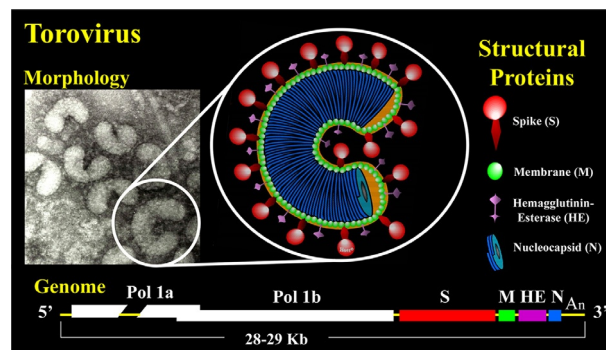


Figure 1 Typical extracellular morphology of a torovirus as visualized by immune electron microscopy (IEM), with a graphic representation of torovirus virion and its structural proteins and genome. Modified with permission from Hoet AE, Torovirus pathogenesis and immune responses. In: Perlman S, Snijder EJ, and Gallagher TM (eds.) *The Nidoviruses*, Copyright 2008 by the ASM Press.

Proteins

Proteins with molecular weights of 20, 22, 37, and 80–100 kDa have been identified in EToV virions. Detergent treatment of virions releases the 22, 37, and 80–100 kDa proteins, which indicates their association with the envelope. Similar polypeptides of 20, 37, 85, and 105 kDa have been identified in purified BToV by means of surface radioiodination. The N protein (167 aa) is the most abundant polypeptide found in the virion (80–84%) with a predicted molecular weight of 18.3–19.2 kDa. Blotting experiments performed with EToV have identified this internal protein as the only RNA-binding polypeptide in the virion. The M glycoprotein (233 aa, 26.5 kDa) is the second most abundant protein (~13%) and is probably associated with the envelope. Computer analysis has revealed characteristics of a class III membrane protein lacking a cleaved signal sequence, but containing three successive transmembrane α -helices in the N-terminal half. The M protein accumulates in intracellular membranes, predominantly those of the endoplasmic reticulum and is believed to play a role in assembly, maturation, and nucleocapsid recognition during the budding process.

The heterogeneous, N-glycosylated 80–100 kDa protein is recognized by both neutralizing and hemagglutination-inhibiting monoclonal antibodies and is therefore identified as the S protein projecting from the virion surface. The S gene encodes an apoprotein (1581 aa) with a MW of about 178 kDa. The deduced amino acid sequence contains domains typical for type I membrane glycoproteins: an N-terminal signal sequence, a putative C-terminal transmembrane anchor and a cytoplasmic tail.

Among the torovirus proteins, the HE protein is relatively well identified. The HE proteins are found in corona-, toro-, and influenza C viruses as well as in infectious salmon anemia virus. The former three HE proteins are thought to be acquired by heterologous RNA recombination; however the source of the HE gene is unknown. The torovirus HE is a class I membrane N-glycosylated protein (416–417 aa) of 65 kDa that is also located in the torovirus envelope. It has an N-terminal signal sequence, a C-terminal transmembrane domain, several N-glycosylation sites and a putative 'FGDS' motif which displays acetyltransferase activity specific for N-acetyl-9-O-acetylneuraminic acid. The HE proteins of BToV and PToV could use N-acetyl-9-O-acetylneuraminic acid as a second receptor. This indicates that the HE protein binds its receptor and in turn cleaves binding between them by itself to enter the cell. EToV virions lack an HE protein, only possessing a partial sequence in ORF4.

Physical Properties

Buoyant densities of 1.16, 1.17, 1.18, and 1.14 g ml⁻¹ have been reported for the virions of EToV, BToV serotype 1, BToV serotype 2, and HToV, respectively. EToV is remarkably stable in the environment, and relatively resistant to phospholipase C, trypsin, chymotrypsin, and even deoxycholate; however, Triton X-100 and organic solvents destroy its infectivity. EToV is stable within a wide range of pH, being inactivated only below pH 2.5 or above pH 10.3. BToV1 appears to be less stable than both EToV and BToV2 as changes in its sedimentation behavior and density have been observed after prolonged storage at -70 °C. The infectivity of a fecal preparation containing BToV1 has been reported to be lost completely after 3 weeks at 4 °C, whereas EToV in cell-free supernatant remained stable for 92 days. Storage of toroviruses at -20° to -70 °C helps to preserve infectivity. However, even at these temperatures the viruses will deteriorate, though at a slower rate. Repeated cycles of freezing and thawing of purified BToV2 results in loss of peplomers and disintegration of virions.

Replication and Morphogenesis

Because it can be grown in cell culture, the morphogenesis of EToV has been most extensively studied. Ultrastructural and immunofluorescence (IF) studies on intestinal cells from BToV-infected calves have shown similarities with EToV morphogenesis.

Attachment, Entry and Uncoating

The attachment to the apical surface of enterocytes is apparently mediated through the spike proteins, but the HE proteins may be involved as well. Entry or penetration of BToV into enterocytes is apparently by receptor-mediated endocytosis. Lysosomal degradation of vesicles containing the virus is probably responsible for uncoating and subsequent release of the BToV RNA. The site at which this occurs has not yet been determined.

EToV replication occurs in the cytoplasm. Preformed tubular capsids bud through membranes of the Golgi stack and of the endoplasmic reticulum. A host cell nuclear function seems to be required since UV preirradiation of cells, actinomycin D, and α -amanitin have been reported to reduce virus yields. The replication cycle takes around 10–12 h to complete.

Transcription

In EToV-infected cells, five virus-specific, polyadenylated mRNAs are found with sizes of >20.0, 7.5, 2.1, 1.4, and 0.8 kb. Northern (RNA) blot hybridizations with restriction fragments from cDNA clones have shown that the five EToV mRNAs form a 3'-coterminal nested set. Sequence analysis has revealed the presence of four complete open reading frames with initiation codons

coinciding with the 5' ends of EToV RNAs 2 through 5, respectively; RNA 5 is contiguous on the consensus sequence. EToV RNAs 1, 2, and 3 are transcribed independently, as has been shown by UV transcription mapping. The genes for M, HE and N are preceded by short non-coding 'intergenic' regions, containing a transcription-regulating element (TRE) conforming to the consensus 5' (C)ACN₃₋₄CUUUAGA 3'. A copy of this sequence is also present at the extreme 5'-terminus of the genome. In contrast, the S gene overlaps with the replicase gene and the N-terminal 28 residues of S are in fact encoded by an internal (-1) reading frame within ORF1b; moreover, there is no TRE. The production of this 3'-coterminal nested set of mRNAs characterizes the toroviruses and justifies their inclusion into the *Nidovirales* order.

Translation

No RNA-dependent RNA polymerase is found in torovirus virions. The torovirus replicase is probably translated as soon as the RNA is liberated. Translation yields two large polyproteins, from which by proteolytic cleavage the various subunits of the viral replicase/transcriptase are derived, as well as accessory proteins of as yet unknown function. Downstream of ORF1b there are the genes for the structural proteins S, M, HE and N (as ordered from 5' to 3'); these are translated from four subgenomic mRNAs, numbered 2 through 5 (with the genomic RNA as RNA 1).

Post-Translational Processing

The *N*-glycosylated spike (S) protein is derived from the processing of a 200 kDa precursor present in infected cells, but not in virions. Eighteen potential *N*-glycosylation sites, two heptad repeat domains, and a possible 'trypsin-like' cleavage site exist in the spike protein amino acid sequence. The mature S protein consists of two subunits and their electrophoretic mobility upon endoglycosidase F treatment suggests that the predicted cleavage site is functional *in vivo*. The heptad repeat domains are probably involved in the generation of an intra-chain coiled-coil secondary structure; similar interchain interactions can play a role in the formation of the observed S protein dimers. The intra- and interchain coiled-coil interactions may stabilize the stalk of the torovirus peplomers.

Assembly, Budding, Egress and Maturation

About 10 h after infection, EToV particles are observed within parts of the unaltered Golgi apparatus, and extracellularly. At that time, tubular structures of variable length, diameter and electron-density appear in the cytoplasm, and also in the nucleus of infected cells, probably representing preformed nucleocapsids. It is unknown whether the accumulation of nucleocapsids in the nucleus reflects a nuclear phase in the replication of EToV or some sort of defective assembly. Viruses predominantly bud into the lumen of Golgi cisternae. The preformed nucleocapsid tubules approach the Golgi membrane with one of the rounded ends oriented towards the membrane and attach to it laterally. During budding, the nucleocapsid is apparently stabilized, leading to a higher electron density and a constant diameter (23 nm). Release into the intestinal lumen is probably through reverse pinocytosis. Virus maturation apparently occurs intracellularly during the egress process, where the virus nucleocapsid appearance changes from a straight rod (intracellular) into a torus shape (extracellular). The characteristic torus morphology of BToV is only observed in extracellular viral particles or in vacuoles near the cell surface, and never in the cytoplasm.

Geographic Distribution

BToV has been detected in fecal samples of cattle by ELISA, RT-PCR, and/or immune-electron microscopy (IEM) in Austria, Belgium, Brazil, Canada, Costa Rica, France, Germany, Great Britain, Hungary, Japan, Korea, the Netherlands, New Zealand, South Africa, and the USA, indicating that they presumably occur worldwide. Sequences analysis showed that Brazilian, European and Asian BToVs have a high degree of identity, but a lower degree of identity with North American strains. Seroprevalence of BToV has also been reported in Belgium, France, Germany, India, Switzerland, United Kingdom, and the USA, ranging between 55% and 94.6%. Most adult horses tested in Switzerland possess neutralizing antibodies to EToV. HToV appears to occur in Brazil, Canada, France, Great Britain, India, the Netherlands and the USA. PToV has been detected by RT-PCR and/or IEM in swine fecal specimens from farms in Austria, Belgium, Canada, China, Hungary, Italy, Korea, South Africa, Spain, the Netherlands and the USA. PToV-seropositive cattle have also been reported in the Netherlands and Switzerland with over 80% seroprevalence. Up to date, TToV has been reported only in the USA.

Host Range

Neutralizing antibodies to EToV have been found in sera from horses, cattle, goats, sheep, pigs, rabbits and feral mice, but not in humans or in carnivores. The host range of BToV appears to be restricted to cattle; however, seropositive reactions to BToV have also been detected in several ungulate species.

TVL particles have been observed in the feces of children and adults with diarrhea. Interestingly, these can be aggregated after the addition of anti-BToV calf sera, can be detected by using a BToV antigen capture ELISA, and their hemagglutination of rat erythrocytes can be inhibited by BToV antisera. These observations indicate antigenic cross-reactivity between HToV and BToV, and may point to a zoonotic connection. TLVs have also been seen in fecal samples of cats and dogs but neither serologic nor molecular identification has been obtained.

Genetic relationships

There is limited genetic information available for toroviruses. The complete genome sequence has been reported only for BToV. However, partial genome sequences have been obtained and used to chart the genetic diversity among toroviruses. In general, there is little divergence (20–40%) among known genotypes of EToV, BToV, PToV, and HToV in the (S, M, HE, and N) genes. Phylogenetic analyses have shown that all BToV strains are closely related, whether they are of European or American origin. Also, all PToV variants form a distinct genetic cluster. However, BToV and PToV sequences are sufficiently different to be assigned as different genotypes. HToV show a high degree of similarity to New World BToVs (83%), and less with European strains of BToV (73%), and PToV (56%).

Antigenic properties

In addition to the typical torovirus morphology, BToV, EToV, HToV, PToV and Lyon-4 virus (Breda-like BToV detected in France) share common antigens. Currently, only one strain of EToV has been isolated, and all attempts to obtain a second equine isolate have been fruitless. Two strains of BToV have been reported in addition to the original isolate described by Gerald Woode and colleagues (1985). One of the strains was detected in feces from a 5-month-old diarrheal calf in Ohio, the second (Iowa strain) was recovered from a 2-day-old experimental animal. On the basis of their cross-reactivity in ELISA, IEM and hemagglutination/hemagglutination inhibition (HA/HI) assays using rat or mouse erythrocytes, the three isolates were assigned to two serotypes: BToV1, represented by the Iowa 1 isolate, and BToV2 comprising the Ohio and the second Iowa isolate.

Antigenic cross-reactivity has been demonstrated by ELISA, IEM, HI and immunoblotting between BToV and HToV, indicating a close relationship. Several authors reported that HToV particles detected in humans with persistent diarrhea, and morphologically similar to BToV, could be agglutinated by BToV antiserum; stronger reactions are observed when BToV-2 antisera are used. Hyperimmune sera to BToV have also been used to detect toroviruses in humans by ELISA and IEM.

Epidemiology

Several epidemiological studies have demonstrated a high seroprevalence of BToV antibodies in several different groups of cattle, indicating that the virus may circulate with high frequency in these populations. BToV have also been detected by ELISA and/or RT-PCR in cases of gastroenteritis in cattle. Up to 44% of the BToV-positive samples from these cases did not contain other major enteric pathogens. Calves up to 4 months of age are highly susceptible to diarrhea induced by BToV, especially those below 3 weeks of age. The virus has also been recognized in 5–6-month-old beef calves arriving from sales barns. Intermittent BToV shedding can occur in young calves during the first 10 months of life. Older calves and adult animals can also shed BToV at different ages, perhaps by intermittent subclinical infections or by contracting new BToV infections. Levels of maternal BToV-specific antibodies circulating in the calf influence the clinical outcome of the infection; a seronegative neonatal calf is about 7 times more likely to develop diarrhea than a seropositive calf.

Epidemiological studies performed in the Netherlands and Spain have also shown a high seroprevalence (81% and 100%) of PToV antibodies in adult sows. Neonates are seropositive by maternal antibody transference through colostrum. After then, its antibody titers decline to close to or below the ELISA cut-off value by the age of weaning. At this point, weaned piglets are infected with PToV and then its antibody titers increase.

HToV is reported to be the principal pathogen in a study examining nurse staffing patterns as well as nosocomial viral gastroenteritis at The Hospital for Sick Children in Toronto, Canada. In particular with nosocomial viral gastroenteritis, HToV is the most commonly identified pathogen (67%), followed by rotavirus in 19%, adenovirus in 9%, norovirus in 4.2% and astrovirus in 1.4%. Children with nosocomial HToV infections are significantly older (median age 3 years) than children with nosocomial rotavirus infections (median age 6 months). Nosocomial HToV infections are more likely to be immunocompromised (50%) than those of rotavirus infection (23%) in children. Nosocomial HToV infections in children have no seasonal influence, while rotavirus infections are prevalent in winter.

Transmission and Tissue Tropism

It has been suggested that the transmission of BToV is via the oral/nasal route by direct contact with contaminated feces or nasopharyngeal secretions. Oral inoculation of calves with BToV has been shown to induce diarrhea with virus shed in feces under experimental conditions. The nasal route is another possible pathway for entry as BToV antigen and viral RNA have been detected in the nasal secretions of feedlot calves. Additionally, diarrhea has been induced after intranasal inoculation in Gn and CD calves. For bovine coronavirus (BCoV), respiratory tract infections have been reported to occur prior to enteric infections, indicating the possible importance of this route of transmission in the spread and pathogenesis of this distantly related group of enteric nidoviruses. It is possible that BToV, like BCoV, could initially replicate in nasal epithelial cells, and thereby amplify before being swallowed and infecting the intestinal tract. This hypothesis of BToV dual tropisms and its role on the virus pathogenesis should be further studied.

BToV has a tissue tropism for enterocytes located from the lower half of the villi extending into the crypts, affecting the caudal portion of the small intestine (mid-jejunum through ileum) and the large intestine. Infection of other types of cells and organs by BToV has not been reported.

Pathogenesis and Clinical Features of Infection

All BToV strains are pathogenic, causing mild to profuse diarrhea in experimentally and naturally infected young calves. Twenty-four to 72 h post-exposure, the first clinical signs are observed (mild fever, depression, weakness, and anorexia), followed by a greenish-yellow to bright yellow watery diarrhea that lasts for 3–5 days. Calves may develop severe dehydration and die. Fecal virus shedding begins 24–72 h post-infection, coinciding with the onset of diarrhea, and lasts for 2–6 days. Shedding peaks around 3–4 days post-infection. Mixed infections with other enteric viruses, such as rotaviruses or astroviruses, result in a more severe watery diarrhea than is induced by either virus alone. In CD calves with a normal intestinal flora, diarrhea is generally more severe than in Gn calves. Sporadic and recurrent shedding of BToV can last for up to 4 months. BToV has also been detected in nasal samples. Further studies are needed to analyze BToV replication and shedding in the respiratory tract as well as its role in respiratory pathologies.

Recently BToV has been associated with outbreaks of epidemic diarrhea in adult cows in dairy farms in Japan, as determined by electron microscopy, RT-PCR and seroconversion (no other pathogen was detected). Affected animals showed diarrhea lasting for 3–5 days with anorexia, decreased milk production and mild respiratory symptoms in some cases. Furthermore, a BToV strain, designated Niigata, was isolated from a fecal sample using HRT-18 cell line.

EToV seroconversion occurs in horses between 10 and 12 months of age, without the appearance of symptoms. Experimentally infected animals (intravenous route) have been reported to seroconvert without clinical signs. Oral infection experiments in horses have not been reported to date. Recently, EToV induces apoptosis in infected cells after peak virus production. This event is mediated by death receptor and mitochondrial pathways involved in the apoptosis process. EToV-induced apoptosis could facilitate viral dissemination *in vivo* and contribute to viral pathogenesis.

Several studies have shown an association of HToV infection with diarrhea in children. In one study, 35% of children with enteritis shed HToVs in their feces, but only 14.5% of the asymptomatic controls shed the virus (statistically significant difference – odds ratio 3.1). Affected children showed watery diarrhea, vomiting 3–4 times daily, and dehydration as a consequence; neither fever, nor the presence of other enteric pathogens was recorded. Recently, fecal excretion of HToV has been associated with nosocomial infections in infants with necrotizing enterocolitis (NEC). Immunocompromised children appear to be highly susceptible to disease following HToV infection.

Pathology and Histopathology

The target organs of BToV in calves are the lower half or two-thirds of the small intestine and the entire large intestine, particularly the spiral colon. There is little macroscopic evidence of the infection. Histological examination shows villous atrophy and epithelial desquamation in randomly scattered areas from the mid-jejunum to the lower small intestine, as well as areas of necrosis in the large intestine. As shown by immunofluorescence, both crypt and villus epithelial cells contain antigen. The watery diarrhea is probably a result of the loss of reabsorptive capacity of the colonic mucosa, combined with malabsorption in the small intestine. The germinal centers of Peyer's patches are depleted of lymphocytes and may occasionally show fresh hemorrhage. The dome epithelial cells, including the M cells, display the same cytopathic changes as seen in the absorptive cells of the villi. Virions are found in cells of both the small and large intestine, and between enterocytes at the basal and lateral plasma membranes. In macrophages of the lamina propria, virions in various stages of degradation are found.

Antigen is detected as early as 48 h after infection in epithelial cells of the lower half of the villi and crypts of the affected areas, as well as in dome epithelium. Fluorescence is cytoplasmic (although a few nuclei may be faintly stained) and generally most pronounced in the intestines with the least tissue damage. The mid-jejunum is infected first, the infection eventually reaching the

large intestine. Diagnosis by IF should be performed preferentially on sections of the large intestine from calves killed after the onset of diarrhea (i.e., several days after infection of the epithelium).

Immune Response

Up to the age of 4 months, all calves in a sentinel experiment regularly excreted BToV in their feces. They showed early serum IgM responses despite the presences of IgG1 isotype maternal antibodies, but no IgA seroconversion. Antibody titers then decreased below detection, persistent IgG1 titers developed in only a few animals. After introduction into the dairy herd at 10 months of age, all calves developed diarrhea and shed virus. Seroconversion for all antibody isotypes was observed at this stage, indicating lack of mucosal memory. In contrast, coronavirus infection in the presence of maternal antibodies leads to isotype switch and a memory response.

Prevention and Control

There are no specific preventive measures for this virus; however, general hygiene, biosecurity practices, and the intake of adequate, protective amounts of colostrum can be used to prevent BToV infections. There are no reports about the effects of disinfection or heat sterilization on toroviruses.

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