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Bovine torovirus (Breda virus) revisited

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Abstract

Bovine torovirus (BoTV) is a pleomorphic virus with a spike-bearing envelope and a linear, non-segmented, positive-sense single-stranded RNA genome. This kidney-shaped virus is associated with diarrhea in calves and apparently has a worldwide distribution. This review provides details of the history and taxonomy of BoTV since its discovery in 1979. Information about virion morphology and architecture, antigenic and biological properties, viral genome, protein composition, thermal and chemical stability, and pH and proteolytic enzymes resistance is also summarized. A major focus of this review is to postulate a possible epidemiological cycle for BoTV, based on epidemiological data obtained in our studies and other published data, and progressing from the newborn calf to the adult animal. The distribution, host range, pathogenesis, disease and clinical signs (under experimental and natural exposure), pathology, diagnosis, prevention, treatment and control of BoTV infections are also described. In addition, a discussion of the zoonotic implications of torovirus-like particles detected in patients with gastroenteritis that resemble and cross-react with BoTV is presented. Hopefully, the findings described here will alert others to the existence of BoTV in cattle and its contribution to the diarrheal disease complex. This review also highlights the need for continual vigilance for potential zoonotic viruses belonging to the order *Nidovirales*, such as the SARS coronavirus.

Keywords: bovine torovirus; Breda virus; epidemiology

Bovine torovirus history and taxonomy

In 1979, an unusual virus with a morphology somewhat distinct from that of known viruses was recognized by electron microscopy in feces from a 5-day-old calf with acute enteritis. No other major enteropathogen was identified in these samples (Woode *et al.*, 1982). The farm was near Breda, Iowa, and up to 56% of the calves developed diarrhea during the first 20 days of life. The newly detected virus was named Breda virus (Woode *et al.*, 1982). In the following years, other strains of Breda virus were identified in calves from Ohio, and Iowa (Woode *et al.*, 1983, 1985).

After the initial reports in cattle, researchers noted the morphological and antigenic similarity of Breda virus to a previously identified equine virus, called Berne virus, which was first described by Marianne Weiss and others (Weiss *et al.*, 1983). Because of their morphological resemblance and serological cross-reactivity, Berne virus and Breda virus were grouped in a provisional family of enveloped RNA viruses, the *Toroviridae* (Horzinek *et al.*, 1984; Horzinek and Weiss, 1984; Horzinek *et al.*, 1987a, b; Weiss and Horzinek, 1987; Ward, 1993). The term 'torovirus' refers to the unique shape of their nucleocapsid in the extracellular environment (Fig. 1) (Horzinek *et al.*, 1987b; Woode, 1990); *torus* is Latin for the circular convex molding in the form of a doughnut shape that some columns or pilasters have in their bases (Weiss and Horzinek, 1987; Woode, 1990; Snijder *et al.*, 1994; Horzinek, 1999).

In April, 1992, a proposal from the *Coronaviridae* Study Group to include *Torovirus* as a genus in the family *Coronaviridae* was accepted by the International Committee on Taxonomy of Viruses (Pringle, 1992;

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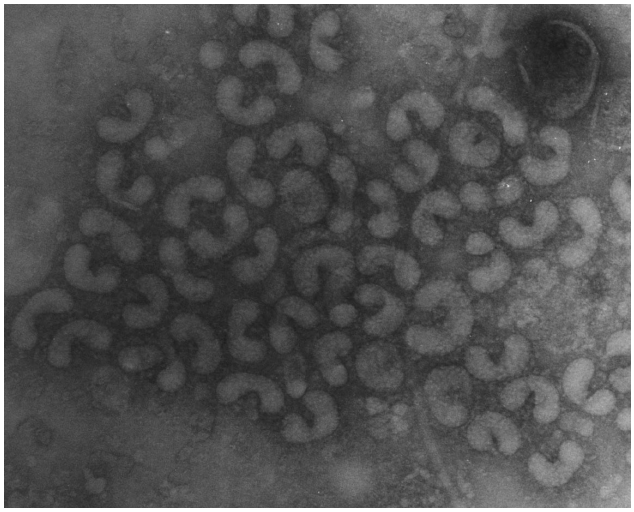

Fig. 1. Electron micrograph of potassium phosphotungstate negative-stained preparations showing the typical morphology of bovine torovirus in feces obtained from a gnotobiotic calf (B-422) 4 days after inoculation. The characteristic kidney shape of the extracellular BoTV and the surface projections or spikes are apparent. A 20% fecal suspension was incubated with hyperimmune antisera to BoTV, resulting in antibody-mediated aggregation of the viral particles. Bar = 100 nm.

Cavanagh and Horzinek, 1993; Horzinek, 1993b; Cavanagh *et al.*, 1994a), and it was later ratified at the IXth International Congress of Virology in 1993 (Cavanagh *et al.*, 1994b). This assignment was based on the morphological and structural similarity among these viruses (Cavanagh, 1997), as well as on other characteristics, such as genomic organization and replication strategies. Based on this classification, the nomenclature reported by Cavanagh *et al.* (1990) for coronavirus genes, mRNAs and structural proteins should also be applied to toroviruses (Cavanagh *et al.*, 1994a).

Four species are recognized in the *Torovirus* genus: the equine torovirus (EqTV), initially known as Berne virus; the bovine torovirus (BoTV), originally known as Breda virus (Cavanagh *et al.*, 1994a; Cavanagh, 1997); the human torovirus (HuTV) (Beards *et al.*, 1984, 1986; Jamieson *et al.*, 1998; Holmes, 2001); and the porcine torovirus (PoTV) (Scott *et al.*, 1987; Kroneman *et al.*, 1998; Holmes, 2001). The EqTV is the prototype virus of the toroviruses and it has been widely characterized because of its ability to grow in cell culture (Snijder and Horzinek, 1993, 1995).

At present the family *Coronaviridae* contains two genera, *Coronavirus* and *Torovirus*, and the family *Coronaviridae* together with the family *Arteriviridae* is grouped under the new order *Nidovirales* (Cavanagh, 1997; de Vries *et al.*, 1997). The order *Nidovirales* is sometimes given the unofficial term 'coronavirus-like superfamily' to indicate their genetic and evolutionary connection of its members (Spaan *et al.*, 1990; Snijder and Horzinek, 1993; Horzinek, 1993a; Snijder *et al.*, 1994; Snijder and Spaan, 1995). Recently, based on comparative sequence analysis, it has been suggested that the *Coronavirus* and *Torovirus* genera should be redefined as two subfamilies within the *Coronaviridae* or two families within the *Nidovirales* (Gonzalez *et al.*, 2003). Extensive reviews of the primary features that characterize the members of the order *Nidovirales* and their relationships are described in the literature (Horzinek and Weiss, 1984; Horzinek *et al.*, 1987b; Weiss and Horzinek, 1987; Spaan *et al.*, 1990; Chirnside, 1992; Horzinek, 1993a, b; Cavanagh and Horzinek, 1993; Cavanagh *et al.*, 1994a, b; Cavanagh, 1997; de Vries *et al.*, 1997; Gonzalez *et al.*, 2003).

Description of bovine torovirus

Virion morphology and architecture

Bovine torovirus is pleomorphic with a spike-bearing envelope. It contains an elongated tubular nucleocapsid, contributing to the structure of the kidney-shaped particle (105–140 nm ×12–40 nm) often viewed in the electron microscope (Saif *et al.*, 1981; Woode *et al.*, 1982; Fagerland *et al.*, 1986; Woode, 1990). The BoTV can be either bald or studded with two types of surface projections (Woode *et al.*, 1982; Lamouliatte *et al.*, 1987; Horzinek and Weiss, 1990; Cornelissen *et al.*, 1997; Cornelissen, 1999). The longer spikes (17–24 nm) have a drumstick- or petal-shaped appearance and are widely spaced in an even manner over the virion surface. They are postulated to correspond to the spike protein (Woode, 1990). The short or smaller surface projections are believed to correspond to the hemagglutinin–esterase protein, which is not present on the EqTV (Woode, 1994; Cornelissen *et al.*, 1997).

The BoTV nucleocapsid is assumed to be a tubular nucleocapsid of helical symmetry that is tightly coiled, forming a hollow tube (resembling the rhabdoviruses) with transverse striations, which can be either straight or bent into an open torus (like a cylinder bending into a ring) (Horzinek *et al.*, 1987a; Horzinek and Weiss, 1990). A tightly fitting lipoprotein membrane or envelope completely covers the nucleocapsid (Horzinek *et al.*, 1987a). The proposed functions of these viral proteins are described in a subsequent section.

Antigenic properties

Woode *et al.* (1983) proposed that BoTV comprises two different serotypes: bovine torovirus serotype 1 (BoTV-1) and bovine torovirus serotype 2 (BoTV-2). This proposal was based on results from several homologous and heterologous antiserum cross-reactivity assays, using tests such as hemagglutination inhibition (HI), immunofluorescence (IF), immune electron microscopy and

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enzyme-linked immunosorbent assay (ELISA). These serological tests showed the presence of common antigens among the different BoTV isolates, but also showed variation among them, indicating the presence of two serotypes. The presence of two distinct serotypes of BoTV was further confirmed by lack of cross-protection in gnotobiotic calf cross-challenge studies (Saif *et al.*, 1981; Woode *et al.*, 1982, 1983, 1985; Lamouliatte *et al.*, 1987). Furthermore, both serotypes of BoTV, as well as EqTV, HuTV, PoTV and Lyon-4 virus (Breda-like BoTV detected in France), share common antigens in addition to their typical morphology (Beards *et al.*, 1984, 1986; Lamouliatte *et al.*, 1987; Weiss and Horzinek, 1987; Woode, 1994). Both serotypes of BoTV posses a hemagglutinin that reacts with erythrocytes from mice and rats, but not with human erythrocytes. The BoTV does not elute from rat erythrocytes after 90 min at 36°C (Woode *et al.*, 1982, 1983, 1985; Koopmans *et al.*, 1986). Because BoTV-1 and −2 do not share common hemagglutination antigens, the HI test has been used to distinguish between the two BoTV serotypes (Woode *et al.*, 1985). Neither of the two BoTV serotypes has shown antigenic cross-reactivity by immune electron microscopy, HI and IF with rotavirus, coronavirus, parainfluenza virus, parvovirus and bovine pestivirus (Woode *et al.*, 1982, 1983, 1985; Lamouliatte *et al.*, 1987; Woode, 1990), nor do they induce cross-protection against bovine coronavirus (BCoV) infection in gnotobiotic calves (Saif *et al.*, 1981).

Biological properties

The BoTV has a tissue tropism for the epithelial cells (enterocytes) of the caudal portion of the small intestine (mid-jejunum to ileum) and the large intestine (Pohlenz *et al.*, 1984; Woode *et al.*, 1985; Woode, 1990). Infection of other types of cells and organs by BoTV has not been reported.

The BoTV does not grow in organ culture, cell culture or in embryonated eggs (Woode, 1990; Horzinek and Weiss, 1990). At present the inoculation of gnotobiotic or colostrum-deprived calves is the only way to propagate this virus. Summarized in Table 1 are the organ cultures, cell cultures and embryonated eggs previously used to try to adapt bovine torovirus to *in vitro* growth, all of which have failed. The strain P138/72 of EqTV is the only torovirus that grows in secondary horse kidney cells, equine dermis cells and embryonic mule skin cells, producing lytic cytopathic effects (Horzinek and Weiss, 1990).

Genome

Toroviruses have a linear, non-segmented, positive sense single-stranded polyadenylated RNA genome about 25–30 kb in length (Weiss *et al.*, 1983; Horzinek *et al.*, 1987a; Snijder *et al.*, 1988; Cornelissen, 1999; Smiths *et*

Table 1 Unsuccessful attempts to adapt bovine torovirus contrasted with the successful attempts to adapt equine torovirus strain P138/72 to *in vitro* growth.

In vitro systems	Treatments	Growth	References
Bovine torovirus			
Primary calf kidney cells (CK cells)	Direct virus inoculation	Negative	Woode *et al.*, 1982
Primary bovine thyroid cells (CTh)	Virus pretreatment with trypsin		
Human rectal tumor cells (HRT18)	Media supplemented with 50?? pig trypsin		
Madin Darby bovine kidney cells (MDBK)			
10-day-old chicken embryos (allantoic route)	Direct virus inoculation	Negative	Woode *et al.*, 1982
Virus pretreatment with trypsin			
Bovine tracheal organ cultures	Direct virus inoculation	Negative	Woode *et al.*, 1982
Primary bovine embryo kidney cells (BEK)	Direct virus inoculation	Negative	Woode *et al.*, 1984
Human rectal tumor cells (HRT18)	Virus pretreatment with pancreatin	Negative	Lamouliatte *et al.*, 1987
Madin Darby bovine kidney cells (MDBK)	Direct virus inoculation	Negative	Vanopdenbosch *et al.*, 1992a
Green monkey kidney cells (VERO)			
Cat kidney cells (CRFK)			
Dog kidney cells (NLDK)			
Pig kidney cells (PK15)			
Swine kidney cells (SK6)			
Secondary fetal calf kidney cells (FCK)			
Calf testicle cells			
Equine torovirus			
Secondary horse kidney cells	Direct virus inoculation	Positive	Weiss *et al.*, 1983
Embryonic mule skin cells (EMS)			
Equine dermis cells (ED)	Direct virus inoculation	Positive	Horzinek and Weiss, 1984
Embryonic mule skin cells (EMS)			
Twenty different established cell lines	Direct virus inoculation	Negative	Horzinek and Weiss, 1984
Embryonic mule skin cells (EMS)	Virus pretreatment with trypsin and β-chymotrypsin	Positive	Weiss and Horzinek, 1986

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al., 2003). The BoTV genome has not been fully sequenced or characterized in journal publications (Koopmans *et al.*, 1991b; Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b; Smiths *et al.*, 2003). However, based on the partial data, as well as that for EqTV, the BoTV genome has six open reading frames (ORFs), with the products of all six known (Snijder and Horzinek, 1993; Smiths *et al.*, 2003). ORFs 1a and 1b are the most 5' reading frames of the torovirus genome and constitute the replicase genes, including the viral RNA-dependent RNA polymerase, which is expressed by a ribosomal frameshift mechanism directly from the genomic RNA (Snijder *et al.*, 1990a; Bredenbeek *et al.*, 1990; Snijder and Horzinek, 1993; Snijder *et al.*, 1994; Smiths *et al.*, 2003).

The other four ORFs encode the structural protein genes and are expressed by the production of a 3'-coterminal nested set of four mRNAs, a characteristic of members of the order *Nidovirales* (Snijder *et al.*, 1990c; Snijder *et al.*, 1994). The ORFs 2–5 encode the spike, membrane, hemagglutinin–esterase and nucleocapsid proteins, respectively (Snijder and Horzinek, 1993; Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b; Smiths *et al.*, 2003). For BoTV, the sequences for ORFs 2–5 are known and they show high homology with EqTV, HuTV and coronaviruses (Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b; Smiths *et al.*, 2003). However, recently the first complete sequence of BoTV-1 was reported at a conference (Draker *et al.*, 2003), including the sequence of ORF1a and 1b (the polymerase gene). Nevertheless, such data are not yet available in GenBank. Additional descriptions of the ORFs relating to their size, sequence, conserved and variable regions, replication strategies, encoded products, and other information have been reported (Spaan *et al.*, 1988, 1990; Snijder *et al.*, 1990a, 1994; Bredenbeek *et al.*, 1990; Snijder and Horzinek, 1993; Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b, 1999; Smiths *et al.*, 2003).

The torovirus genome has intergenic sequences or motifs (conserved AU-rich areas) preceding ORFs 2–5, which are presumptive transcription initiation sites that direct the synthesis of subgenomic mRNAs (Snijder and Horzinek, 1993; Snijder *et al.*, 1994; Cornelissen *et al.*, 1997, 1999; Duckmanton *et al.*, 1998b; Cornelissen, 1999). Also, at the 3' end of the BoTV genome there is a non-coding or non-translated region between the polyadenylated tail and ORF 5, and such a sequence may play a role in the initiation of virus replication (Duckmanton *et al.*, 1998b).

Proteins

Snijder and Horzinek (1993) described the protein composition of toroviruses, using as a model the EqTV, and suggested that the viral proteins of BoTV might be similar. Recent research has confirmed that BoTV basically has the same viral proteins as EqTV (Koopmans *et al.*, 1986; Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b). Several published articles characterize in detail the torovirus proteins (Koopmans *et al.*, 1986; Zanoni *et al.*, 1986; Horzinek *et al.*, 1986; Snijder *et al.*, 1989, 1994; Woode, 1990; Den Boon *et al.*, 1991; Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b; Kroneman *et al.*, 1998; Cornelissen, 1999).

The nucleocapsid (N) protein is the most abundant polypeptide found in the virion, and represents almost 80–84% of the total protein mass (Horzinek *et al.*, 1987b; Horzinek and Weiss, 1990). The N proteins are internal to the virion, and this protein is the only RNA-binding polypeptide found in virions from infected cells (Horzinek and Weiss, 1990; Duckmanton *et al.*, 1998b). The amino acid sequences of the N protein in EqTV, BoTV and PoTV have been published (Kroneman *et al.*, 1998).

The membrane (M) glycoprotein has been previously referred to as the matrix or envelope protein. This glycosylated protein is the second in abundance representing about 13 % of the virion protein mass (Koopmans *et al.*, 1986; Cavanagh *et al.*, 1990; Den Boon *et al.*, 1991; Snijder and Horzinek, 1993; Duckmanton *et al.*, 1998b). This protein is probably associated with the envelope and it has been suggested to play a role in the assembly, maturation, and nucleocapsid recognition during the budding process (Horzinek and Weiss, 1990; Snijder *et al.*, 1994; Duckmanton *et al.*, 1998b), but its immunogenicity is low (Koopmans *et al.*, 1986).

The spike (S) glycoprotein, previously called the peplomer protein, comprises the envelope projections or spikes of toroviruses, and may be present as a dimer of two subunits (Horzinek and Weiss, 1990; Cavanagh *et al.*, 1990; Snijder and Horzinek, 1993; Cornelissen, 1999). The spike configuration of the BoTV-1 S protein is stabilized by a coiled-coil secondary structure, which gives a golf club shape to the S protein (Duckmanton *et al.*, 1998b). The predicted S protein of BoTV has a cleavage site for a trypsin-like protease (Snijder *et al.*, 1990b; Duckmanton *et al.*, 1998b). It is believed that the S protein is involved in the receptor binding process and viral infectivity, together with hemagglutinating activity (Zanoni *et al.*, 1986; Horzinek *et al.*, 1986; Cornelissen, 1999); however, these spikes can be lost after high speed centrifugation (80000–100 000 *g*) (Woode, 1990). Additionally, some authors have speculated that the epitope(s) responsible for the cross-reactivity between the different BoTV serotypes and EqTV are located on the S protein, to which both neutralizing and HI antibodies are directed (Beards *et al.*, 1986; Koopmans *et al.*, 1986; Lamouliatte *et al.*, 1987; Horzinek and Weiss, 1990; Koopmans and Horzinek, 1994).

The hemagglutinin–esterase (HE) glycoprotein is located in the BoTV envelope (Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b). The HE has a putative F–G–D–S motif, which displays acetylesterase activity specific for N-acetyl-9-*O*-acetylneuraminic acid. This catalytic site is conserved among the HE proteins of

coronaviruses, influenza C virus and BoTV. The precise function of the HE protein of toroviruses is unknown; however, it has been suggested to function as a receptor-binding and receptor-destroying protein (Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b; Holmes, 2001). On the other hand, some authors have indicated that the HE protein could have a potential role in viral migration through the mucus layer that protects the enterocytes (Cornelissen *et al.*, 1997). It is important to highlight that the EqTV does not have HE proteins on the virion (Cornelissen *et al.*, 1997; Duckmanton *et al.*, 1998b).

The toroviruses produce their own viral replicase (RNA-dependent RNA polymerase) in a highly conserved manner for all three members of the *Nidovirales* (Snijder and Horzinek, 1993). No RNA polymerase is found in torovirus virions; therefore, the torovirus replicase is immediately translated inside the infected cells (Snijder and Horzinek, 1993; Cornelissen, 1999; Holmes, 2001). Like coronaviruses and other positive-strand RNA viruses, the torovirus replicase has two conserved domains, the RNA-dependent RNA polymerase and the NTP-dependent helicase (Koonin, 1991; Snijder *et al.*, 1994). However, based on the unusual organization of these domains in the coronavirus and torovirus polymerase, they were classified as an outgroup in the supergroup I positive-strand RNA viruses (Koonin, 1991).

Thermal and chemical stability, pH and proteolytic enzymes resistance

The EqTV is inactivated at a linear rate between 31° and 43°C, which makes it more easily heat-inactivated than some coronaviruses, such as transmissible gastroenteritis virus of swine (Weiss and Horzinek, 1986a; Horzinek and Weiss, 1990). When fecal samples are stored at 18–25°C, the morphological characteristics and the hemagglutinin specificity of the BoTV are stable for at least 10 days. However, at temperatures above 4°C the infectivity of the virus in fecal samples is lost within 24–48 hours (Woode, 1990). If the BoTV is stored at 4°C, it has an appreciable loss of infectivity around 92–185 hours, losing the total infectivity after 2–3 weeks. Therefore, it is necessary to freeze the virus at temperatures between –20° and –70°C to keep it stable and preserve the infectivity; however, the virus will still deteriorate, but at a slower rate (Koopmans *et al.*, 1986; Horzinek and Weiss, 1990; Woode, 1990; Koopmans and Horzinek, 1994). The poor preservation of the virus will affect the physical and chemical properties of BoTV. It was reported that BoTV-2 is relatively more stable than BoTV-1 during prolonged storage (Koopmans *et al.*, 1986). Loss of spikes and disintegrated virions was reported when repeated cycles of freezing and thawing were applied to BoTV (Koopmans and Horzinek, 1994), as also observed in our laboratory.

Regarding the chemical stability of BoTV, treatment with chloroform or diethyl ether induces the loss of infectivity, as expected for an enveloped virus. In general, the toroviruses are very resistant to phospholipase C, trypsin and chymotrypsin, and the two latter enzymes actually enhanced EqTV infectivity (Weiss and Horzinek, 1986a; Horzinek and Weiss, 1990; Cornelissen, 1999). No specific information is available about BoTV pH resistance; however, EqTV has a wide range of pH stability, being inactivated only below pH 2.5 or above pH 10.3 (Horzinek and Weiss, 1984, 1990; Weiss and Horzinek, 1986a).

Epidemiology

The epidemiological cycle of BoTV

From epidemiological data obtained in our studies, plus previously published information, it is possible to assemble a plausible epidemiological cycle for BoTV (bold numbers and letters cited in text refer to Fig. 2). Calves [**1**] from a few days up to 4 months old are susceptible to diarrhea induced by BoTV, but infections occur more frequently between 2 and 5 days of age (Woode *et al.*, 1982; Durham *et al.*, 1989; Koopmans *et al.*, 1990, 1991c; Woode, 1990; Hoet *et al.*, 2003b). However, the course of BoTV infection will differ, as for many enteric viral diseases, depending on colostrum intake to obtain passive immunity. Therefore, a neonatal calf without maternal immunity [**2**] (seronegative or with very low titers of BoTV antibodies) is apparently highly susceptible to BoTV infection (odds ratio, 6.95) during its first month of life (Hoet *et al.*, 2003c). This calf may develop moderate to severe diarrhea associated with BoTV infection and generate an active immune response that can be measured by HI (Hoet *et al.*, 2003c). The detailed patterns of IgG1, IgM and IgA antibody responses to BoTV have been described elsewhere (Koopmans *et al.*, 1990; Koopmans and Horzinek, 1994).

On the other hand, if a calf acquires maternal immunity [**3**] to BoTV, it will be partially protected against disease produced by this virus, having mild or no diarrhea. However, the calf could still shed low amounts of BoTV in feces without the presence of diarrhea (subclinical infection), and in some cases no seroconversion is detected by HI up to 21 days after infection (Hoet *et al.*, 2003c). Similar scenarios have been observed in other studies of BoTV, as well with other members of the family *Coronaviridae*, such as the BCoV. The presence of passive (IgG_1) serum antibodies may not block viral shedding and is associated with decreased or delayed active immune responses in infected calves (Woode *et al.*, 1985; Weiss and Horzinek, 1987; Woode, 1990; Koopmans *et al.*, 1990, 1991c; Heckert *et al.*, 1991a, b; Koopmans and Horzinek, 1995).

After the initial infection, and if the calf survives, the affected calf [**4**] may be fully protected or it can intermittently shed BoTV at different ages up to 6 months or

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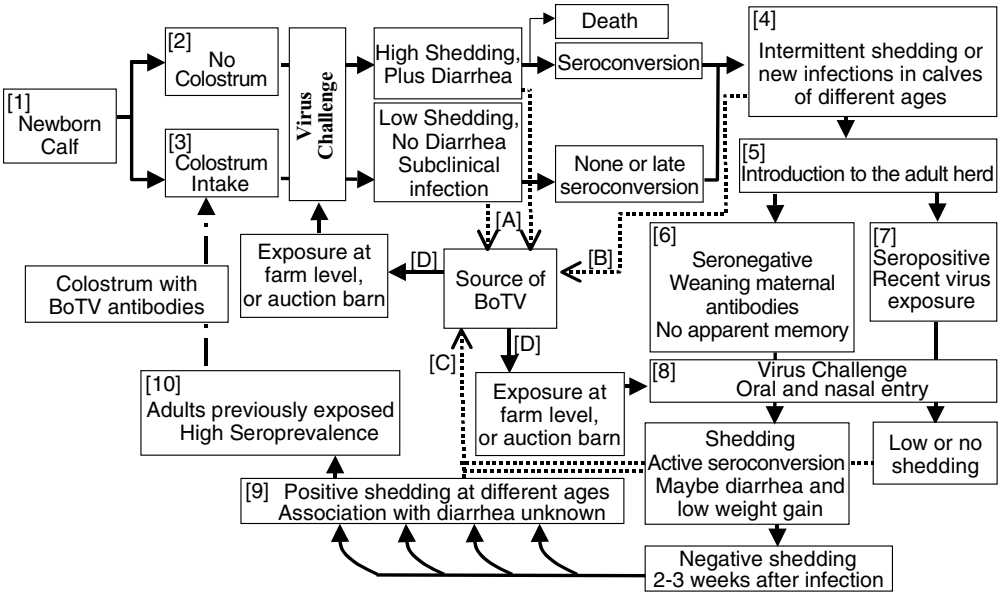

Fig. 2. An overview of bovine torovirus epidemiology. The potential epidemiological cycle for BoTV is described step by step, starting with a newborn calf and progressing to the adult animal.

more. In support of this assumption are the observations of our study (Hoet *et al.*, 2003b), in which BoTV shedding was detected in several young calves from 1 to 7 months old. Similar findings were also reported for sentinel calves, which shed BoTV intermittently during the first 10 months of life, becoming an important source of BoTV [**B**] (Koopmans *et al.*, 1990; Koopmans and Horzinek, 1995).

Diarrheic calves aged 1 month or less that are shedding BoTV are apparently one of the most important sources of BoTV [**A**]. These young calves are apparently highly susceptible to BoTV infection, as reported previously, whereby up to 71% of the BoTV-positive-samples submitted to the diagnostic laboratory came from calves less than 3 weeks old (Hoet *et al.*, 2003b), making this group the principal source of BoTV. Other possible sources of BoTV [**C**] are older calves and adult animals shedding BoTV at different ages, perhaps by intermittent subclinical infections or by contracting new BoTV infections. This assumption is based on the fact that this virus has also been recognized in 5- to 7-month-old beef calves arriving from sales barns and entering feedlots (Saif *et al.*, 1981; Hoet *et al.*, 2002); in calves up to 10 months old, where several of them have been reported to develop diarrhea associated with BoTV lasting for 1 to 3 weeks (Koopmans *et al.*, 1990); and in yearling and adult cows (Woode, 1990). The latter source was also confirmed in our study (Hoet *et al.*, 2003b), in which up to 28% of the BoTV-positive samples submitted to the diagnostic laboratory came from adults older than 2 years. Although there are no reports about reservoirs for BoTV (Woode, 1990), all sources of BoTV indicated above can be the origin of BoTV infections [**D**] transmitted to highly susceptible young calves, as well as to older calves with declining or lack of maternal immunity.

Once a calf reaches the age of 5–7 months, it usually enters the adult herd or a feedlot [**5**]. Then, it can face six major scenarios (Fig. 3), which have two primary outcomes: seronegativity (<10–20 Hemagglutination Inhibition Units, HIU) or seropositivity (>40 HIU) to BoTV. As in neonatal calves, the presence of circulating antibodies to BoTV will affect the final outcome when an older calf is exposed to BoTV. It is unknown for how long a calf retains circulating antibodies to BoTV after an active immune response. However, whatever maternal immunity a young calf obtains will disappear around 5–6 months of age. This statement is supported by the finding that almost no 5- to 7-month-old feedlot calves (93%) in our feedlot study (Hoet *et al.*, 2002) had antibodies to BoTV at their arrival on the farm. This natural decline of maternal antibodies to BoTV was also observed in other studies, where most of the calves tested between 2 and 6 months old became seronegative to BoTV (Koopmans *et al.*, 1989, 1990). In addition, some authors (Koopmans and Horzinek, 1994) have suggested that BoTV-infected young calves have a deficient or lack of mucosal immunological memory, which may be due to the apparent damage of BoTV infection to M cells, and/or to the lack of an active antibody response to BoTV due to interference by maternal antibodies (Koopmans *et al.*, 1989, 1990; Koopmans and Horzinek, 1994).

If the calf arrives into a feedlot or herd with no (seronegative) or very low circulating antibodies to BoTV [**6**], it apparently will have a high susceptibility to BoTV infections. In a feedlot study (Hoet *et al.*, 2002) of the BoTV seronegative calves at arrival, 100% subsequently shed BoTV fecally and/or nasally and

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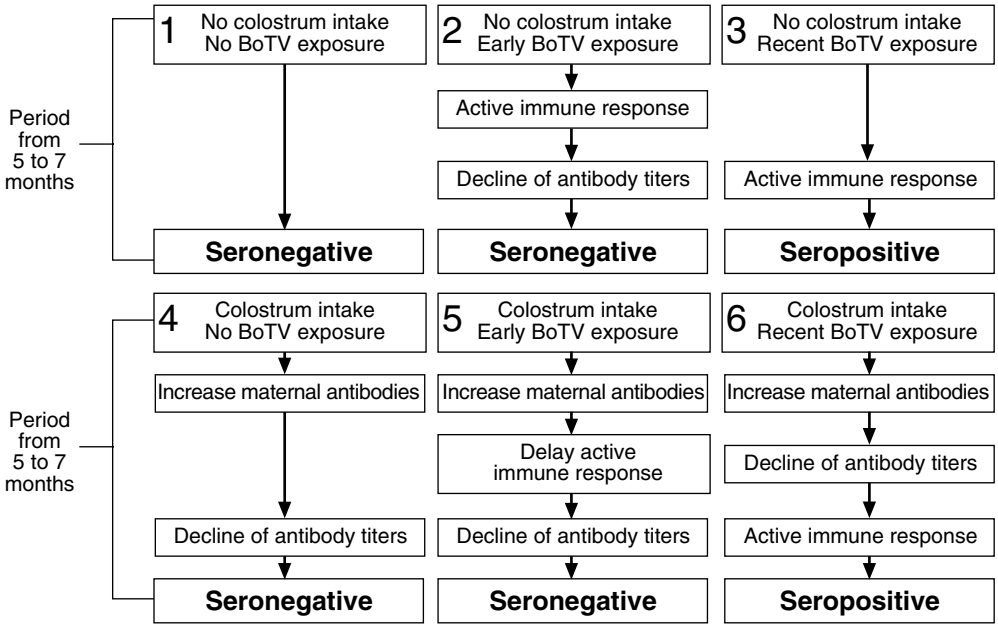

Fig. 3. Six major BoTV infection scenarios that a 5- to 7-month-old calf could face during its development, all of which have two primary outcomes before the calf enters an adult herd or feedlot. Seronegativity (≤0–20 HUI) or seropositivity (≥40 HUI)

seroconverted to BoTV (by HI). Similar findings reported previously coincide with this observation, whereby seronegative calves seroconverted to BoTV when they were placed in contact with adult cows (Koopmans *et al.*, 1989). Koopmans *et al.* also reported seroconversion associated with all antibody isotypes tested and BoTV shedding associated with mild diarrhea in seronegative older calves when they were mixed with adult cattle apparently infected subclinically with BoTV (Koopmans *et al.*, 1990). Therefore, the lack of circulating antibodies, together with the mixing of animals, the stressful conditions and exposure to BoTV shedders, can predispose an incoming seronegative calf entering an adult herd or a feedlot to new BoTV infections, which might induce diarrhea and affect the performance of this calf. Nevertheless, after the initial infection and production of antibodies, which occurs approximately 21 days after infection, all of the affected calves in the feedlot study recovered and stopped shedding BoTV. Fecal and nasal shedding patterns of BoTV in feedlot cattle at the time of arrival and periodically throughout the first 21 days after arrival at a feedlot are described elsewhere (Hoet *et al.*, 2002).

On the other hand, if [**7**] the arriving calf is seropositive with moderate to high levels of circulating antibodies to BoTV (> 40 HIU), the calf would be apparently 'protected' against a new BoTV infection, as was observed for the seropositive calves arriving at a feedlot (Hoet *et al.*, 2002). They did not shed detectable virus and their pre-existing antibody titers did not increase. The antibodies to BoTV in these calves at arrival were probably from an active immune response to a recent or continuous BoTV exposure.

The transmission of BoTV has been suggested to be through the oral/nasal route by direct contact with contaminated feces or nasopharyngeal secretions. In the 6- to 7-month-old calf [**8**] illustrated, transmission could be through the oral route, as has been proven under experimental conditions in which oral inoculation of calves with BoTV-induced diarrhea with fecal viral shedding (Woode *et al.*, 1982, 1984, 1985; Woode, 1990). The nasal route is another possible pathway for BoTV entry. This assumption is based on the detection of BoTV antigen and viral RNA in the nasal secretions of almost every feedlot calf, on at least one sampling time-point during the study (Hoet *et al.*, 2002). The BCoV were previously associated with the bovine respiratory disease complex (i.e. shipping fever) (McNulty *et al.*, 1984; Kapil and Goyal, 1995; Storz *et al.*, 1996, 2000a, b; Martin *et al.*, 1998; Lathrop *et al.*, 2000; Lin *et al.*, 2000; Chouljenko *et al.*, 2001). In addition, respiratory tract infections by BCoV prior to enteric infections have been reported, which indicates the possible importance of this route of transmission in the spread and pathogenesis of BCoV infections and the production of enteric and respiratory disease (Reynolds *et al.*, 1985; Saif *et al.*, 1986; Heckert *et al.*, 1990, 1991c; Hasoksuz *et al.*, 1999; Cho *et al.*, 2001). Therefore, it is possible that BoTV, like BCoV, replicates initially in the nasal epithelial cells, increasing the virus titers before being swallowed and infecting the intestinal tract (Saif *et al.*, 1986). This initial replication in the upper respiratory tract would increase the opportunity for survival of these types of enveloped viruses during passage through the stomach and into the gastrointestinal tract. Although BoTV antigen and viral RNA have been detected in nasal samples (Hoet *et al.*, 2002), nasal repli-

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cation and its contribution to the pathogenesis of BoTV need to be examined further. Also, possible damage to the upper or lower respiratory tract and aerosol transmission among susceptible animals should be assessed.

Data from our studies showed [**9**] that diarrheic older heifers (>7 months old) and diarrheic adult cattle shed BoTV throughout the year; however, its association with diarrhea in these cattle populations is still unclear (Hoet *et al.*, 2003b). Nevertheless, the shedding of BoTV by subclinically, chronically or intermittently infected animals agrees with the assumption that the prevalence of this virus is widespread in the cattle population, as judged by the high seroprevalence of BoTV antibodies (by ELISA) reported in the USA (ranging from 88.5 to 89.7%) (Woode *et al.*, 1985; Woode, 1987, 1994). One consequence of the high seroprevalence of BoTV antibodies in the adult population [**10**] is the high number of neonatal calves (≤7 days old) with antibodies to BoTV, related to colostrum intake. Eighty-three per cent of the neonatal calves included in our veal study (Hoet *et al.*, 2003c) had antibodies to BoTV at their arrival at the farm. A similar seropositivity rate (90%) was observed previously in calves less than 1 month old (Koopmans *et al.*, 1989). These maternal antibodies, as described earlier, will probably affect the course of the BoTV infections in neonatal calves.

Distribution

BoTV has been detected in the USA, specifically in Iowa, Ohio and South Dakota (Saif *et al.*, 1981; Woode *et al.*, 1982, 1985; Lamouliatte *et al.*, 1987; Woode, 1990; Hoet *et al.*, 2002, 2003b), as well as in other countries worldwide such as Belgium (Vanopdenbosch *et al.*, 1991, 1992a,b), Canada (Durham *et al.*, 1989; Duckmanton *et al.*, 1998a), Costa Rica (Pérez *et al.*, 1998), France (Lamouliatte *et al.*, 1987), Germany (Kluver, 1991; Liebler *et al.*, 1992), Great Britain (Brown *et al.*, 1987), Netherlands (Koopmans *et al.*, 1991c), New Zealand (Koopmans and Horzinek, 1995), South Africa (Vorster and Gerdes, 1993) and Hungary (Matiz *et al.*, 2002).

Seropositivity to toroviruses in cattle has been also reported in other parts of the world, such as Belgium, France, Germany, India, Switzerland and the UK (Brown *et al.*, 1987; Cornelissen, 1999). In the UK, Brown *et al.* (1987) found a high prevalence (55%) of antibodies to BoTV in cattle. Similarly, in The Netherlands and Germany, up to 94.6% of the cattle tested were seropositive by ELISA to BoTV-2. These findings suggest endemic BoTV infections in these regions (Koopmans *et al.*, 1989).

Host range

The host range of BoTV appears to be restricted to cattle; however, toroviruses are apparently widespread in ungulates, since sera from horses, cattle, sheep, goats and pigs have shown high percentages of seropositive animals (Weiss *et al.*, 1984; Horzinek and Weiss, 1984, 1990; Horzinek *et al.*, 1987b; Woode, 1990). Torovirus-like particles have also been detected in other animals species, such as pigs (Scott *et al.*, 1987; Durham *et al.*, 1989; Lavazza and Perini, 1990; Penrith and Gerdes, 1992; Matiz *et al.*, 2002), turkeys (Ali and Reynolds, 1997, 1998, 2000), dogs (Hill and Yang, 1984; Finlaison, 1995) and cats (Muir *et al.*, 1990).

Morphogenesis

The morphogenesis of toroviruses has been principally described using EqTV as the prototype, because of its ability to grow in cell culture (Weiss and Horzinek, 1986b). Ultrastructural studies using electron microscopy and IF on intestinal cells from BoTV-infected calves showed similarities with EqTV morphogenesis (Pohlenz *et al.*, 1984; Woode *et al.*, 1985; Fagerland *et al.*, 1986). However, many steps in the BoTV replication process are still unknown. This topic has recently been reviewed in more detail (Hoet, 2002).

Pathogenesis of bovine torovirus infection

The pathogenesis of the disease has been described in colostrum-deprived and gnotobiotic calves from 3 to 50 days of age (Saif *et al.*, 1981; Pohlenz *et al.*, 1984; Woode *et al.*, 1984, 1985; Fagerland *et al.*, 1986). Once BoTV is inoculated orally or intranasally, it rapidly infects the epithelial cells from the lower half of the villi, extending into the crypts throughout the mid-jejunum, ileum, colon and cecum; this results in diarrhea 24–72 hours after challenge without presenting a viremic phase (Woode *et al.*, 1982, 1984, 1985; Pohlenz *et al.*, 1984; Fagerland *et al.*, 1986; Hall, 1987; Woode, 1990). In some cases, BoTV antigen has been detected in dome epithelial cells and M cells, in which cytopathic changes are observed (Pohlenz *et al.*, 1984; Woode *et al.*, 1984). Some authors indicate that BoTV only infects absorptive enterocytes, because the virus had not been observed in other cell types (Fagerland *et al.*, 1986). However, Woode *et al.* (1982) suggested that the first site for viral replication could be the immature epithelial cells of the crypts, from which the infection could migrate up the villi (Koopmans and Horzinek, 1995).

The cytopathic effects produced by BoTV on epithelial cells have been described (Woode *et al.*, 1982, 1984, 1985; Pohlenz *et al.*, 1984; Fagerland *et al.*, 1986; Hall, 1987; Woode, 1990). By a few hours after infection, the enterocytes show signs of severe vacuolar degeneration, necrosis and exfoliation, all of which induce villous atrophy, crypt hyperplasia and, in some cases, fused villi. In the colon (principally the spiral colon) and cecum, the

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BoTV infects the surface and crypt epithelial cells, where similar lesions have been observed (Pohlenz *et al.*, 1984; Woode *et al.*, 1984). Some authors (Hall, 1987) suggested that the infection and necrosis of both villi and crypt enterocytes in the lower small intestine, as well as surface and crypt enterocytes in the large intestine, appeared to be unique to BoTV.

The mechanism by which BoTV induces diarrhea is unknown; however, Woode *et al.* (1982) speculated that watery diarrhea could be the product of the colonic lesions produced by the virus and the consequent reduction in the ability to absorb water. In addition, the damage to the villi and crypt enterocytes could produce a malabsorptive/maldigestion effect to induce diarrhea. This last assumption is based on the fact that BoTV produces a 15–65% reduction in the absorption rate of D-xylose (Woode *et al.*, 1985; Woode, 1987). Woode also indicated that 30–50% of the upper small intestine appears normal or without lesions, which could explain why the BoTV diarrhea is only mild to moderate (Woode *et al.*, 1985; Woode, 1987). Although BoTV antigen and viral RNA have been detected in nasal samples (Hoet *et al.*, 2002), there is no information on the pathogenesis of BoTV infections in the upper and lower respiratory tract.

Disease and clinical signs

Bovine torovirus has been described as an enteric pathogen that causes mild to profuse diarrhea in experimentally or naturally infected young calves. In the following section, the most relevant findings about the disease produced by BoTV infections under experimental and natural conditions will be described.

Experimental infection

It has been proved that BoTV-1 and −2 can induce diarrhea in gnotobiotic and colostrum-deprived calves (Saif *et al.*, 1981; Woode *et al.*, 1982, 1984, 1985; Woode, 1982; Pohlenz *et al.*, 1982, 1984; Tzipori, 1985; Fagerland *et al.*, 1986). The disease and clinical signs produced by BoTV in experimentally infected calves (3–50 days old) can be summarized as follows. Twenty-four to 72 hours after challenge the calf shows a moderate increase in body temperature (39.4–40°C), depression, weakness and anorexia. A few hours later, greenish-yellow soft feces are observed, changing from a dark brown to greenish-yellow or bright yellow stool of watery consistency. The diarrhea is the principal sign of disease and usually lasts 3–5 days. The calf may develop severe dehydration in a period of 24–48 hours, and 48–96 hours after of the onset of diarrhea the calf may die (Woode *et al.*, 1982, 1984, 1985; Pohlenz *et al.*, 1984; Woode, 1990).

In experimental animals, BoTV fecal shedding began 24–72 hours after infection coinciding with the onset of diarrhea, and lasted for 2–6 days (Woode *et al.*, 1982; Woode, 1990). In our experience (Saif *et al.*, 1981), fecal shedding of BoTV can last up to 6–7 days, with the peak shedding around 3–4 days after inoculation. L. J. Saif (unpublished communication) reported fecal shedding of BoTV up to 10–14 days after challenge in 3- to 4-week-old gnotobiotic and colostrum-deprived calves by using immune electron microscopy for detection.

Also in experimental infections, mixed inoculation with BoTV and other enteric viruses, such as rotavirus and astrovirus, produced a more severe watery diarrhea than the clinical disease induced by either of the viruses alone, suggesting that these viruses might have an additive effect in the induction of diarrhea (Woode *et al.*, 1984, 1985). Another interesting point postulated by Woode *et al.* (1982) was that BoTV may interact with the microbial flora in the intestine to induce the full clinical effect. This hypothesis was based on the observation that only one-quarter of the gnotobiotic calves were severely affected compared with 100% of the colostrum-deprived calves with a normal flora.

Natural exposure

Studies of several outbreaks of diarrhea related to BoTV in cattle farms, as well as epidemiological studies showing the apparent association between BoTV and diarrhea, have been published (Woode *et al.*, 1982; Lamouliatte *et al.*, 1987; Koopmans *et al.*, 1990, 1991c; Scott *et al.*, 1996; Pérez *et al.*, 1998; Duckmanton *et al.*, 1998a). In our laboratory, several studies have been done to determine the association between BoTV shedding and diarrhea under field conditions. In a study conducted during a 19-month period, BoTV was detected in 9.7% of samples from clinical cases of gastroenteritis in cattle from Ohio, principally in young calves less than 3 weeks of age. Interestingly, 44% of the BoTV-positive samples did not contain other major enteric pathogens, and BoTV was found only in the diarrheic and not in the healthy adult cattle (Hoet *et al.*, 2003b). In a follow-up study in a veal farm, a significant independent association (odds ratio, 6.95) between BoTV shedding and diarrhea was observed in calves less than 15 days old (Hoet *et al.*, 2003c). The results of both studies provide supporting evidence that BoTV may be an important pathogen of neonatal and young calves, and could be involved in the acute undifferentiated diarrhea in neonatal calves.

In the first natural outbreak reported, calves between 2 and 20 days of age (average 3–5 days old) showed 'a yellow to white semisolid or watery diarrhea, profuse in quantity and inducing severe dehydration' (Woode *et al.*, 1982). Additionally calves showed weakness, dehydration, depression and varying degrees of anorexia and

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fever 24–48 hours after the diarrhea onset (Woode, 1987). Similarly, J. L. Saif (unpublished communication) reported that 4- to 5-day-old calves were affected by BoTV in a field outbreak, showing mild diarrhea and signs similar to those previously reported.

In naturally infected calves, BoTV shedding can be detected in feces preceding and during clinical signs, in subclinical cases, and in sporadic and recurrent shedding that can last up to 4 months (Woode, 1990; Koopmans *et al.*, 1990). Woode *et al.* (1987) noted that the shedding of BoTV in calves under field conditions varied considerably both in duration of virus excretion and in virus titer produced; therefore, the isolation rates of BoTV in feces under field conditions may be low.

Calves with respiratory disease signs during BoTV outbreaks have also been described (Koopmans *et al.*, 1989; Hoet *et al.*, 2002). Vanopdenbosch *et al.* (1992a,b) reported respiratory toroviral infections in young calves from 4 days up to 6 months of age, detected by immunofluorescence. However, the reagents used by Vanopdenbosch *et al.* were suspected of being contaminated with BCoV antibodies; therefore, their results should be interpreted with caution (Cornelissen *et al.*, 1998). In our feedlot study, BoTV antigen and viral RNA were detected in nasal secretions from animals with respiratory disease; however, we did not detect an association between BoTV shedding and clinical disease (Hoet *et al.*, 2002). Further studies are needed to analyze BoTV replication and shedding in the respiratory tract to determine the role that BoTV may play in respiratory tract infections.

Some diarrheic adult cows have shown various degrees of seroconversion to BoTV; therefore, BoTV has been suspected, like BCoV in outbreaks of winter dysentery (Koopmans *et al.*, 1989, 1991c; Van Kruiningen *et al.*, 1992). In one of these studies, farms with dairy cows seroconverting to BoTV were 3.5 times more likely to have a winter dysentery episode than farms with cows without seroconversion (Koopmans *et al.*, 1991c). However, other studies indicate a major role of BCoV in winter dysentery causation (Smith *et al.*, 1998a,b) rather than BoTV. Thus, the serological evidence for the role of BoTV in the winter dysentery disease syndrome is inconclusive and needs to be explored further (Koopmans *et al.*, 1989; Van Kruiningen *et al.*, 1992).

Pathology

Macroscopic or gross lesions

Few macroscopic changes have been observed in BoTV-infected animals. However, clear signs of dehydration may be evident at necropsy, together with reddening and loss of tone of the thin-walled lower small intestine (Pohlenz *et al.*, 1982; Woode, 1990; Koopmans and Horzinek, 1994).

Microscopic lesions (histopathology)

Villous atrophy with focal necrosis of epithelial cells covering the lower 50% of the villus and extending deeply into the crypts was observed in randomly scattered (patchy) areas from the mid-jejunum to the ileum and from the surface of the large intestine into the deep folds (specially in the colon). In addition, mild to moderate inflammatory responses were observed, principally in the small intestine, where the crypts were dilated and contained a large amount of cell debris and abundant infiltration of macrophages and neutrophils in the lamina propria (Saif *et al.*, 1981; Woode *et al.*, 1982, 1984, 1985; Pohlenz *et al.*, 1982, 1984; Fagerland *et al.*, 1986; Hall, 1987; Woode, 1990). Infection and destruction of both crypt and villous epithelial cells of the small intestine appears to be a unique characteristic of BoTV (Woode *et al.*, 1982; Pohlenz *et al.*, 1984; Fagerland *et al.*, 1986; Hall, 1987). Intracellularly, BoTV particles are observed as elongated tubules with rounded ends (35–42 × 80–105 nm) and are found in the cytoplasm and nucleus of infected intestinal cells (Pohlenz *et al.*, 1984; Fagerland *et al.*, 1986).

Diagnosis

BoTV can be detected in fecal samples from a few hours before the onset of clinical symptoms, during the presence of diarrhea, and up to several days after the feces return to normal (Saif *et al.*, 1981; Woode *et al.*, 1982; Woode, 1990). Bovine torovirus can also be detected in nasal swabs at the same time that the enteric shedding occurs (Hoet *et al.*, 2002). Because BoTV does not grow in cell culture or any other *in vitro* system, it is necessary to apply other techniques for its diagnosis. BoTV has been detected in fecal samples, small and large intestinal contents, nasal swabs and tissues by electron microscopy and immune electron microscopy in negative-stained samples (Saif *et al.*, 1981; Woode *et al.*, 1982; Woode, 1987, 1990; Hoet *et al.*, 2003a), ELISA to detect viral antigen and BoTV antibodies (Boom, 1986; Koopmans *et al.*, 1989, 1993b; Hoet *et al.*, 2003a), hemagglutination/hemagglutination inhibition test (HA/HI) (Woode *et al.*, 1982; Woode, 1987; Hoet *et al.*, 2003)a, immunofluorescence (Woode, 1987, 1990; Horzinek and Weiss, 1990; Koopmans and Horzinek, 1994), nucleic acid hybridization (dot blot system) (Koopmans *et al.*, 1991b; Duckmanton *et al.*, 1998b) and reverse transcriptase–polymerase chain reaction (RT-PCR) (Duckmanton *et al.*, 1998a; Hoet *et al.*, 2003a). Extraction of toroviral RNA from intestinal tissue sections preserved in various fixatives for subsequent RT-PCR amplification has been also described (Koopmans *et al.*, 1993a). Monoclonal antibodies against EqTV have been developed and their properties studied (Kaeffer *et al.*, 1989); however, their cross-reactivity with BoTV has not been studied. There is no information about monoclonal

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antibodies against BoTV and their potential use in diagnostic assays. The design, standardization and validation, as well as the advantages and disadvantages of some of those diagnostic techniques, have been described and summarized recently (Hoet *et al.*, 2003a). Additionally, details about processing nasal samples and the detection of BoTV by ELISA and RT-PCR have also been published (Hoet *et al.*, 2002).

Prevention, treatment and control

There are no specific preventive measures for this virus (Horzinek and Weiss, 1990); however, general hygiene and biosecurity practices should be applied to reduce outbreaks of BoTV. Antibody-containing colostrum (500 ml/day) can also be used as prophylaxis against BoTV infections (Woode, 1990). Young calves may need fluid therapy to recover during the diarrhea; however, older calves usually survive the disease without treatment if secondary infections are not present (Koopmans *et al.*, 1990). There are no reports about the effects of disinfection or heat sterilization on BoTV; however, Woode indicated that heat, disinfection or desiccation should easily destroy the virus (Woode, 1990).

Human torovirus

In 1984, torovirus-like (ToVL) particles resembling bovine torovirus were detected in feces from patients with gastroenteritis by using electron microscopy (Beards *et al.*, 1984). Since then, multiple reports of toroviruses in humans have been published (Beards *et al.*, 1986; Brown *et al.*, 1987, 1988; Lacombe *et al.*, 1988; Koopmans *et al.*, 1991a, 1993b, 1997; Tellier and Petric, 1993; Koopmans and Horzinek, 1995; Middleton, 1996; Duckmanton *et al.*, 1997; Krishnan and Naik, 1997; Jamieson *et al.*, 1998). The TVL particles have been detected principally in children and adults with acute diarrhea in several countries, including the USA, France, The Netherlands, Canada, Great Britain, India and Brazil (Beards *et al.*, 1984; Lamouliatte *et al.*, 1987; Lacombe *et al.*, 1988; Koopmans *et al.*, 1993b, 1997; Krishnan and Naik, 1997). Based on the supporting evidence presented in the last few years, the term 'human torovirus' (HuTV) is now used to describe the TVL particles detected in human fecal samples (Koopmans and Horzinek, 1994; Duckmanton *et al.*, 1997).

Beards *et al.* (1986) proposed five criteria to identify and report HuTV particles, whereby a positive finding should meet at least three of these criteria: (i) the mean size of the viral particle should be around 100 nm; (ii) it should bear spikes approximately 10 nm long on the surface; (iii) it should have a torus shape; (iv) its buoyant density in sucrose should be 1.14–1.16 g/ml; and (v) the particles should be agglutinated by antisera to EqTV or BoTV. This last characteristic is very interesting, because apparently there is a close relationship between BoTV and HuTV, whereby antigenic cross-reactivity has been demonstrated by ELISA, immune electron microscopy, HI, immunoblotting and nucleic acid hybridization (cDNA probes) (Beards *et al.*, 1984, 1986; Woode, 1990; Koopmans *et al.*, 1993b; Duckmanton *et al.*, 1997).

There are several studies describing the prevalence of HuTV and its apparent association with diarrhea. In one study, toroviruses (Jamieson *et al.*, 1998) were detected by electron microscopy in 35% of children with gastroenteritis and in only 14.5% of the asymptomatic controls. The difference between the two groups (odds ratio, 3.1) was statistically significant. In many cases, HuTV was also the only enteropathogen identified, strongly supporting the suggestion that toroviruses could have a causative role in the induction of nosocomial enteric infections in immunocompromised and older children (Jamieson *et al.*, 1998). In Ceará, Brazil, HuTV was significantly associated (22%, by ELISA and electron microscopy) with both acute and persistent (chronic) diarrhea in children under 18 months of age (Koopmans *et al.*, 1997). It is important to highlight that although HuTV was present alone only in two of those cases, HuTV was not detected in the healthy control group (Koopmans *et al.*, 1997). Interestingly, in this study acute diarrhea was commonly associated more with torovirus than with rotavirus (Koopmans *et al.*, 1997). Thus, more studies of HuTV are needed, especially regarding their relationships to BoTV.

We hope that the findings here presented will alert others to the existence of BoTV in the cattle population and its contribution to the diarrhea disease complex. Further studies of BoTV epidemiology are needed to assess BoTV prevalence, its role in the production of disease, and its effect on performance. The outcome of such future research and ours should ultimately lead to improved health and production of our food-producing animals, and to understanding of the zoonotic potential of BoTV in the human population. Certainly, the unexpected appearance of SARS coronavirus in humans, probably transmitted from animals, highlights a need for continued vigilance in investigating the zoonotic potential of additional members of the *Nidovirales*.

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