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The Ecobiology of Toroviruses (*Tobaniviridae*) and Their Association with The Enteric Diseases in Animals and Humans

Mohd Iswadi Ismail*

Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia

***Email**: iswadi@dvs.gov.my

Abstract

Torovirus (ToV) is one of the responsible for causing gastroenteritis diseases in animals and humans worldwide. Since its discovery in the 1970s, ToVs remained one of the important causative agents for gastroenteritis and diarrhoea in horses, cattle, sheep, goats, pigs, and other animals including humans. Therefore, the ecobiology and association of ToVs in animals and humans remained to be elucidated. The molecular basis of ToVs in animals and humans has been well described. ToVs can be recognised as enveloped viruses with non-segmented and positive-sense of RNA genome, and pleomorphic shape. In addition, the recent epidemiology, clinical signs, diagnostic methods, and suitable prevention and control measures are also explained in this review. Clinical signs of ToV infection are vary; these include pyrexia, diarrhoea, dehydration, lethargy, depression, and death. Various diagnostic methods were used in the detection of ToVs such as electron microscopy, RT-PCR, haemagglutination inhibition, ELISA, and neutralisation assay. At present, no vaccine is available for ToV, but the virus can be prevented by applying good hygiene, strict biosecurity, and proper sanitary conditions.

Keywords

Torovirus, Animal, Human, Enteric disease

Introduction

The toroviruses (ToVs) are classified within the genus *Torovirus*, family *Tobaniviridae*, and order *Nidovirales* (ICTV, 2021). The family tree of ToVs is presented in Figure 1. Various studies reported that the ToVs are pathogens responsible for causing gastroenteritis in animals and humans (Dai et al., 2021; Ujike & Taguchi, 2021; Hu et al., 2019; Dhama et al., 2014).

Morphologically, ToVs are spherical, oval, elongated, or kidney-shaped enveloped viruses with a single-stranded RNA genome of positive polarity (Figure 2). The virus resembles coronaviruses (CoVs) in genome organisation and replication strategy but differs in virion architecture, especially the nucleocapsid (N), which is a tubular structure responsible for the singular particle morphology (Hoet, 2008).

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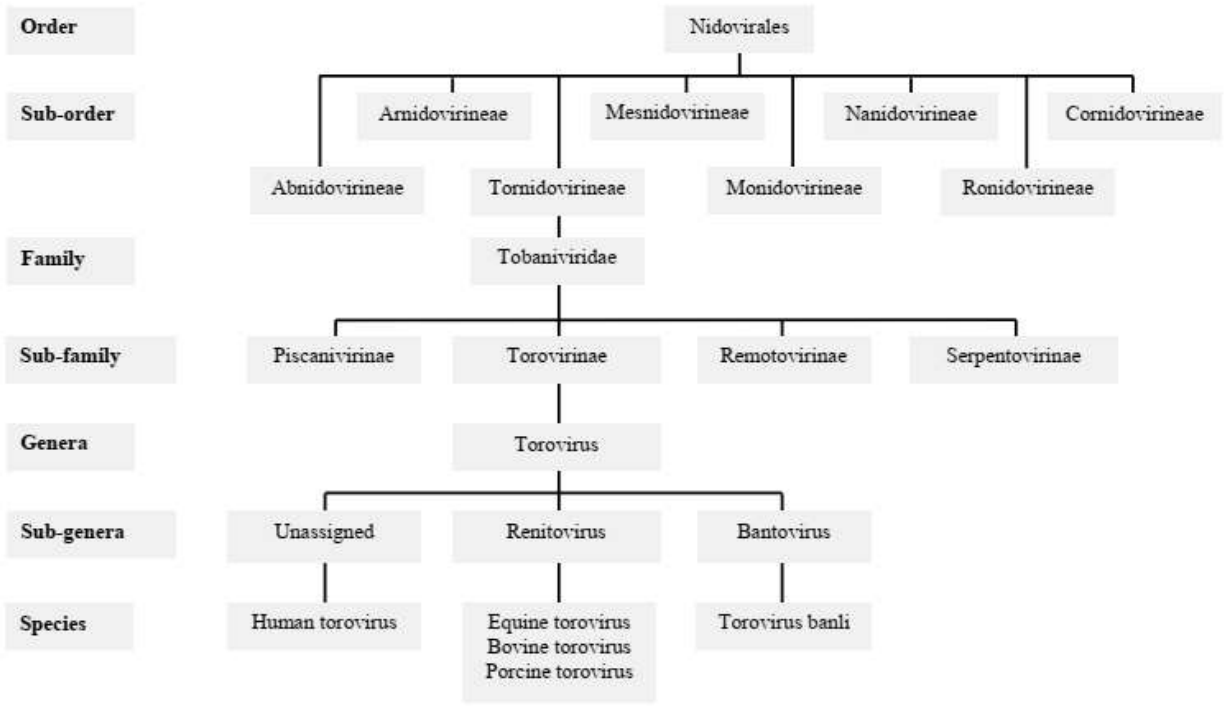

Figure 1. Family tree of toroviruses (described by Schoch et al., 2020)

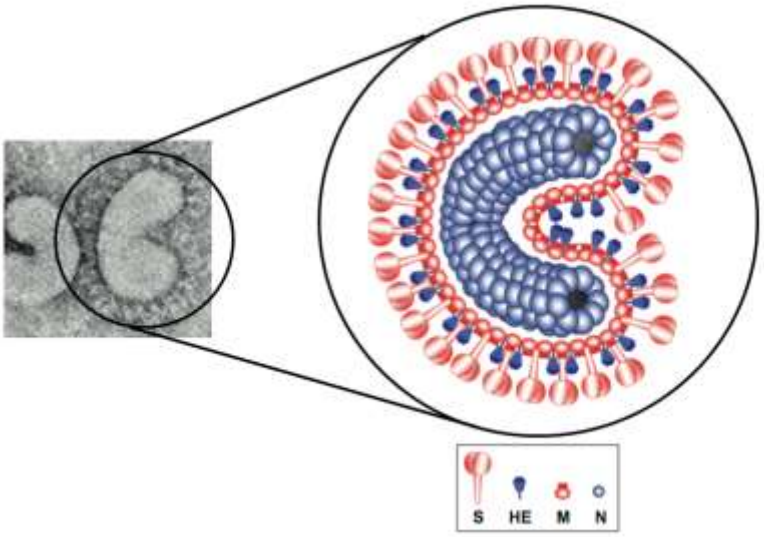

Figure 2. Schematic representation of typical extracellular morphology of the ToV virion. The structural proteins of the spike (S), haemagglutinin-esterase (HE), membrane (M), and nucleocapsid (N) are indicated (adapted from ICTV, 2011).

The ToVs appear to occur worldwide and torovirus-like (TVL) particles in faecal preparations have been reported from different regions including Europe, America, Asia, New Zealand, and South Africa (Anbalagan et al., 2014; Petric, 2003). This review introduces the ToVs regarding their discovery and classification, morphological characteristics, genetic structure and

variation, replication and host range, recent epidemiology, diagnostic methods, and possibilities for future research.

Torovirus Aetiology and Classification

Toroviruses are enveloped, single-stranded, positive-polarity RNA viruses with a peplomer-bearing envelope (ICTV, 2021). The term *torus* (Latin) refers to the circular convex moulding in the form of a doughnut that some columns or pilasters 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 (Horzinek, 1999). The general features of ToV are presented in Table 1.

Table 1. General features of toroviruses (reviewed by Cho & Hoet, 2014)

Features	Descriptions
Buoyant densities	
EToV	1.16 g ml^{-1}
BToV 1	1.17 g ml^{-1}
BToV 2	1.18 g ml^{-1}
HToV	1.14 g ml^{-1}
Unique biconcave disk and C-shape virion	105-140 x 12-40 nm
Enveloped	+, 11 nm thick
Elongated tubules with rounded ends	35-42 x 80-105 nm
Genome size (kb)	28.5 kb
Positive-polarity ssRNA genome with peplomer-bearing envelope	+
5’ polymerase gene-structural protein genes 3’	+
3’ co-terminal nested set of ≥4 subgenomic mRNAs	+
Polymerase gene has two ORFs, 1a and 1b	+
The 1b ORF is translated after ribosomal frameshifting	+
M protein has three membrane-spanning sequences	+
Virion formation at internal membranes	+
5’ leader sequence	+
Nucleocapsid	+, Helical symmetry coiled into a hollow tube
Prominent S glycoprotein	+, 8-10 nm
Prominent HE protein (class I membrane N-glycosylated protein)	+

+, the feature present in the virus

Since 1992, the genus *Torovirus* has been included with the genus *Coronavirus* and the newly recognised genus *Bafinivirus* in the family *Coronaviridae,* based on similarities in genomic organisation and replication strategies. Toro- and coronaviruses are also ancestrally related; their polymerase and envelope genes diverged from those of a common predecessor (Hoet, 2008). Because of their inclusion in the family *Coronaviridae,* the nomenclature for coronavirus genes, mRNAs, and structural proteins have also been applied to ToVs. 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 (Horzinek, 1999).

Recently, the International Committee on Taxonomy of Viruses (ICTV) presently recognises five species in the genus *Torovirus*: *equine torovirus* (EToV), *bovine torovirus* (BToV), *porcine torovirus* (PToV), *human torovirus* (HToV), and *Torovirus banli* (ICTV, 2021). The *Torovirus banli* is a tick-borne viral pathogen; therefore it will be not reviewed in this paper. Interestingly, a previous study revealed that there is antigenic cross-reactivity relatedness between EToV, BToV, PToV, and HToV (Smits et al., 2003). Moreover, the genetic divergence among the viruses is about 20 to 40% (Cho, 2014).

Genomic Organisation

The ToV genome is single-stranded, nonsegmented, polyadenylated RNA of positive polarity and 25 to 30 kb in size. The genome contains six open reading frames (ORFs), which are transcribed as a 3′ co-terminal nested set of four mRNAs (Figure 3).

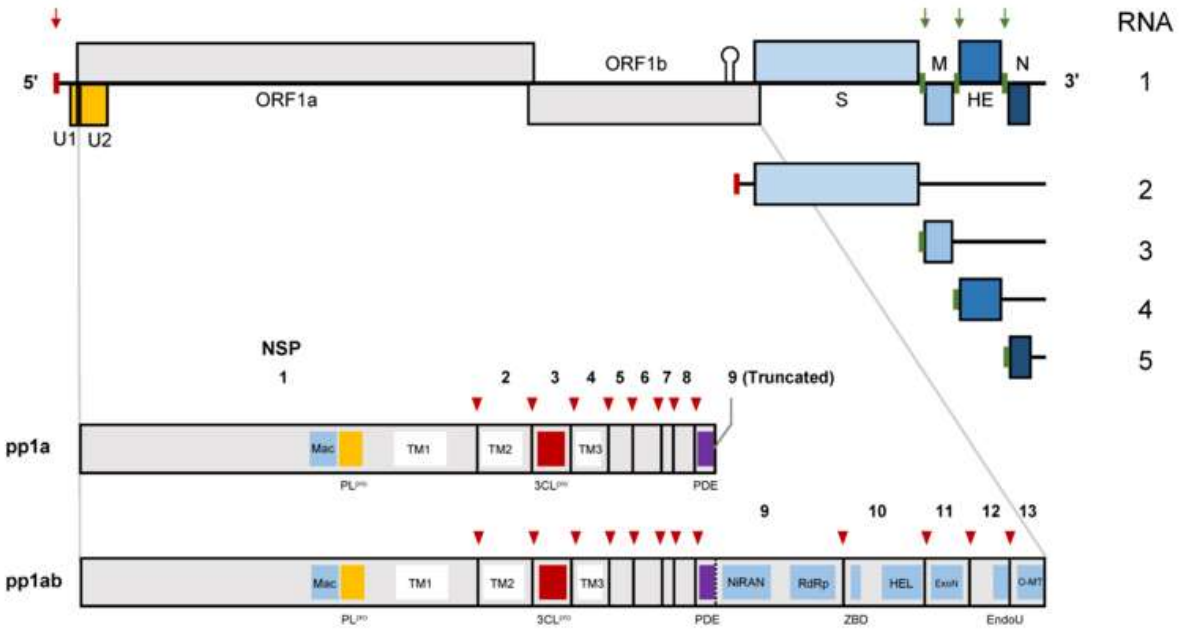

Figure 3. Schematic genome organisation of ToV (adapted from Ujike & Taguchi (2021)

Legend: U, unconventional CUG initiation codon; ORF1a, open reading frame 1a; ORF1b, open reading frame 1b; S, spike; M, membrane; HE, haemagglutinin esterase; N, nucleocapsid; RNA, ribonucleic acid; pp1a, polyprotein 1a, pp1b, polyprotein 1b, NSP, non-structural protein; PL^{PRO}, papain-like protease; $3CL^{PRO}$, 3C-like protease; EndoU, endoribonuclease; ExoN, exoribonuclease; HEL, helicase, Mac, macrodomains including ADP-ribose-1"phosphatase; NiRAN, nidovirus RdRp-associated nucleotidyltransferase; N-MT, guanosine N7-methyltransferase; O-MT, ribose 2'-O-methyltransferase; PDE, 2',5'-phosphodiesterase; RdRp, RNA-dependent RNA polymerase; TM, transmembrane domain; ZBD, zinc-binding domain.

The genome has 5'-UTR and 3'-UTR, respectively. Its 5′ two-thirds are occupied by two large overlapping open reading frames, ORF1a and ORF1b. The ORF1a and -1b encode polyproteins from which the viral polymerase, RNA-dependent RNA polymerase (RdRp) is derived (Draker et al., 2006). The polymerase is involved in the synthesis of a negative-strand RNA and the onset of genomic and sub-genomic RNA synthesis. In a larger view, these polyproteins are proteolytically processed into 13 non-structural proteins (nsps) (Ujike & Taguchi, 2021).

Downstream of ORF1b are four smaller ORFs, ORF 2, 3, 4, and 5 encode the structural proteins; spike protein (S), integral membrane protein (M), class I membrane protein haemagglutinin-esterase (HE) exhibiting acetylesterase activity, and nucleocapsid protein (N) as shown in Table 2 (Cho & Hoet, 2014).

Table 2. Virion-associated proteins of toroviruses (described by Cho & Hoet, 2014).

Proteins	**Size (kDa)**
Spike glycoprotein (S)	178
Integral membrane protein (M)	26.5
Haemagglutinin-esterase protein (HE)	65
Nucleocapsid protein (N)	18.3-19.2

Replication

The replication of ToV started with the attachment of the S and HE proteins to main host receptors, mostly a specific glycoprotein and mediates entry of ToV into the host cells via endocytosis (Table 3). Evidence shows that S proteins and HE proteins act cooperatively for ToV binding to the host cells (Sriwilaijaroen & Suzuki, 2020). The entry of the viral genome into the host cell will trigger the signalling pathways. Concomitantly, the virus produces RdRP which is involved in the synthesis and proteolytic cleavage before the fusion of the virus membrane. Then the virus starts to release its genomic ssRNA(+) into the host cell cytoplasm. Using the genomic ssRNA(+) as a template, the dsRNA genome is synthesised. The dsRNA is transcribed to provide the viral mRNA genomes. These sub-genomic mRNAs encode the structural proteins. At the end of the replication, the virus assembles in the endoplasmic reticulum (ER), the intermediate compartments, and the Golgi complex before budding at the membranes of the cell and will be released as a new virion.

Table 3. Cellular binding receptors of the spike (S) and haemagglutinin-esterase (HE) proteins of toroviruses

References	Viruses	Cellular binding receptors	
		S protein	HE protein
Sriwilaijaroen & Suzuki, 2020; Langereis et al., 2009; Smits et al., 2005	EToV	9-mono-*O*-acetylated sialic acids	5-*N*-acetyl-9-*O*-acetylneuraminic acid
Sriwilaijaroen & Suzuki, 2020; Smits et al., 2005	BToV	9-mono-*O*-acetylated sialic acids	5-*N*-acetyl-7(8),9-di-*O*-acetylneuraminic acid

Sriwilaijaroen & Suzuki, 2020; Langereis et al., 2009; Smits et al., 2005	PToV	9-mono-*O*-acetylated sialic acids	4,9-di-*O*-acetyl-Neu5Ac-
Sriwilaijaroen & Suzuki, 2020; Langereis et al., 2009; de Groot, 2006; Smits et al., 2005	HToV	9-mono-*O*-acetylated sialic acids	5-*N*-acetyl-9-*O*-acetylneuraminic acid

Host Range

The ToVs are species-specific; however recent studies reported cross-species infections with one or a few ToVs. Previous studies reported that the EToV, BToV, PToV, and HToV have been found in horses, cattle, porcine and humans, respectively (Smits et al., 2003). Recently, several studies reported that the neutralising antibodies to EToV have been found in sera from horses, cattle, goats, sheep, pigs, rabbits, and feral mice, but not in humans or carnivores. On the other hand, the neutralising antibodies to BToV showed that the host range of BToV appears to be restricted to cattle. Interestingly, seropositive reactions to BToV have also been detected in several ungulate species (i.e.; horses, sheep and goats).

Cross-reacting antibodies were also reported in the serological diagnosis of PToV. Cross-reactivity was reported in the neutralising assay to PToV using anti-EToV sera (Kroneman et al., 1998). In addition, cross-reactivity to BToV was detected using PToV sera in the ELISA method.

The HToV is detected in faecal samples of diarrheic children and adults (Sriwilaijaroen & Suzuki, 2020). However, HToV has also been reported in faecal samples of cats and dogs but neither serologic nor molecular identification has been obtained. Other studies showed that the HToV can be aggregated after the addition of anti-BToV calf sera, and then detected by using a BToV antigen capture ELISA. Furthermore, the haemagglutination of rat erythrocytes by HToV can be inhibited by adding the BToV antisera. These observations indicate antigenic cross-reactivity between HToV and BToV and may point to a zoonotic connection.

Transmission

The ToVs can be transmitted via the oral or nasal route by direct contact with contaminated faeces or nasopharyngeal secretions (Hoet, 2008; Hoet & Saif, 2008).

Support in other studies that oral inoculation of calves with BToV has been shown to induce diarrhoea with virus shed in faeces under experimental conditions (Hoet et al., 2002). 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. Therefore, the BToV suggested enter the respiratory tract before causing enteric infections, indicating the possible importance of this route of transmission. Possibly, BToV could initially replicate in nasal epithelial cells, and thereby amplify before being swallowed and infecting the intestinal tract.

In addition, 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 and large intestines (Hoet et al., 2003b). Infection of other types of cells and organs by BToV has not been reported.

Pathogenicity

Different pathogenicity of ToVs is reported. For example, the EToV is infecting horses, causing severe diarrhoea. Interestingly, EToV is the only ToV that has been grown in vitro.

On the other hand, all BToV strains are pathogenic, causing diarrhoea in either experimentally or naturally infected calves (Castells & Colina, 2021). The infection with PToV in porcine is generally subclinical; however, the signs included diarrhoea, anorexia, dehydration, lethargy, and depression (Qin et al., 2021).

HToV has been associated with gastroenteritis and diarrhoea in humans, especially in children. In addition, HToV was reported to cause nosocomial infections in infants with necrotising enterocolitis (Hoet & Saif, 2008).

Toroviruses of Veterinary Important

Toroviruses were found to play a key role as a causative agent of gastroenteritis in a range of animal families of equine, bovine and porcine (Koopmans & Horzinek, 1994; 1995).

Equine torovirus

In 1972 the first torovirus, equine torovirus (EToV, also known as Berne virus) was accidentally isolated in equine kidney cells from a rectal swab taken from a diarrhoeal horse at the University of Berne, Switzerland (Sneijder & Horzinek, 1993; Koopmans & Horzinek, 1994). Interestingly, the EToV is the only torovirus that has been successfully propagated in cell culture (Maestre et al., 2011).

In Europe, the seroprevalence of EToV-specific antibodies is very high in the horse population with 35% of randomly collected sera in Germany and 81% in Switzerland being positive (Petric, 2003).

In addition, the EToV was reported not neutralised by antisera against different known equine viruses. Serologic cross-reactions were observed in neutralisation tests and ELISA using sera from calves that had been experimentally infected with morphologically similar particle, and then named ‘Breda’ viruses (later named bovine torovirus, BToV). Thus, the findings indicate that the EToV and BToV were found to be antigenically related (Smits et al., 2003).

Bovine torovirus

The bovine torovirus (BToV, also known as Breda virus), was discovered in 1979 during an investigation in a dairy herd in Breda, Iowa, USA, causing severe diarrhoea in neonatal calves (Hoet et al., 2003b; Hoet & Saif, 2007). The calves generally develop symptoms of diarrhoea, typically lasting 2 to 13 days (Koopmans et al., 1991). Despite repeated attempts, BToV cannot be adapted to grow in cell or tissue cultures and must be passaged in gnotobiotic (Gn) calves (Woode et al., 1982).

Two serotypes of BToV have been identified namely BToV-1 and BToV-2. The BToV-1 serotype represents the original BToV isolate from Breda, Iowa, whereas, the BToV-2 serotype represents the BToV isolate from colostrums-deprived calves in Iowa, and isolate from diarrheic calves in Ohio (Woode et al., 1985).

Surveillances in the 1980s reported that cattle sera collected were found to have a high prevalence of seropositive BToV antibody in the United Kingdom (55%) (Brown et al., 1987), and in the USA (88.5%) (Woode et al., 1985) to BToV when examined in ELISA. On the other hand, surveillance in 2000s reported that in Japan, BToV was detected at 15.2% (15 out of 99) (Kirisawa et al., 2007), and in Turkey, BToV was detected at 16.7% (12 out of 72) (Aydin et al., 2019) from diarrhoeic faecal samples. Recently, a low prevalence rate of BToV at 1.74% (8 out of 461) from diarrhoeic faecal samples was reported in China (Shi et al., 2020).

The pathogenicity of BToV infections is mainly in the enteric tract, but the virus can also affect the respiratory tract causing respiratory diseases. The thinness in the intestinal wall is usually observed during gross examination. In addition, during the histopathological examination, fusion and atrophy of the villi and epithelial desquamation are observed from the middle jejunum to the lower small intestine as well as in areas of necrosis in the large intestine (Castells & Colina, 2021). Uniquely, the BToV infection can be identified by detecting the necrosis of the villi and crypt enterocytes in the small intestine (other gastroenteritic viruses usually infect the villi cells but not the crypt cells). The BToV reported is often observed in multiple, large autophagolysosomes (Pohlenz et al., 1984).

Porcine torovirus

The porcine torovirus (PToV, also known as porcine Markelo virus) was first reported in 1998 in Hargeerds Farm, Markelo, the Netherlands (Kroneman et al., 1998). Since then, the virus were reported to emerge in many countries around the world. The PToV was reported as the causative agent for enteric disease in porcine in Canada, the USA, South Africa, China, Korea, the UK, Italy, Belgium, Hungary, and Spain (Alonso-Padilla et al., 2012).

The infection of PToV is generally asymptomatic (Hu et al., 2019), however, the co-infections with other porcine pathogens may lead to unpredictable outcomes.

Two lineages of PToV have been identified, namely Markelo and P4. The Markelo and P4 strains share an amino acid sequence homology of 80% (Pignatelli et al., 2013). Interestingly, both PToV strains carry different HE proteins, which suggested the immune response generated against

one PToV strain did not protect the porcine against the infection by the other strain. Moreover, the sera tested by haemagglutination inhibition (HI) assay and ELISA revealed antigenic differences between the two lineages.

Toroviruses Associated with the Human Enteric Tract

In 1984, torovirus-like (TVL) particles were first detected in the diarrheic faeces from human patients with gastroenteritis, which was later named human torovirus (HToV). Since then, reports of HToVs in humans, especially children and adults with acute diarrhoea have appeared in several countries (Jamieson et al., 1998). Hence, studies report evidence that HToVs are associated with gastroenteritis in humans.

The HToVs are detected from diarrheic faeces of children; 27% (9 out of 33) children with acute diarrhoea, and 27% (11 out of 41) children with persistent diarrhoea (Koopmans et al., 1997). In another study, HToV was detected in 35% (72 out of 206) of gastroenteritis cases in children (Jamieson et al., 1998). Clinically, humans that are infected with HToV were more frequently immunocompromised (43% cases) and nosocomially infected (58% cases). They may experience less vomiting (47% cases) but had bloody diarrhoea (11% cases).

Diagnostic Assays

Diagnosis of ToVs usually uses sera or faecal samples. The ToVs can be detected in faecal samples from a few hours before the onset of clinical symptoms, during the presence of diarrhoea, and up to several days after the faeces return to normal (Hu et al., 2019). In addition, the virus can also be detected in nasal swabs at the same time that enteric shedding occurs in infected animals (Hoet et al., 2002).

Different methods or assays were used to detect ToVs. These include conventional and advanced methods. In the early years, immunoelectron microscopy was used to detect ToVs (Duckmanton et al., 1997). However, the disadvantage of this method includes high cost, time-consuming and it is not suitable to be used to process a large number of samples. Moreover, the polymorphisms of ToV decrease the specificity and accuracy of the method. Then the PCR was used as a diagnostic assay for detecting the ToVs (Hoet et al., 2003b; Hosmillo et al., 2010). The methods become a method of choice because it is low-cost, convenient, highly specific, and sensitive. Conventional RT-PCR and quantitative RT-PCR (qRT-PCR) are the most commonly used in detecting the ToVs. Besides, advanced PCR such as nested PCR, multiplex RT-PCR, and RT-LAMP is also used (Hu et al., 2019).

Different serological assays were applied for the detection of antibodies from the sera of EToV, BToV, PToV, and HToV such as haemagglutination inhibition (Shimabukuro et al., 2013), neutralisation assay (Pignatelli et al., 2009), and ELISA (Dhama et al., 2014). Indirect ELISA was a method of choice for serological diagnosis because of its high sensitivity in detecting the virion, however, the specificity of the method may be slightly worse (Hoet et al., 2003a). Therefore,

practically, the combination of ELISA and RT-PCR is suggested to be used in ToVs diagnosis (Dhama et al., 2014).

Prevention and Control

There are no specific preventive measures for ToVs infection; however, general hygiene, biosecurity practices, and the intake of adequate, protective amounts of colostrum can be used to prevent ToVs infection (Cho & Hoet, 2014). Moreover, there is no specific information available for ToVs control. Hence, there is no vaccine available for ToV. In addition, there are also no reports on the effects of disinfection or heat sterilisation on ToVs (Dhama et al., 2014).

Conclusions

Although in most cases stool specimens obtained from animals with diarrhoea were examined, the pathogenic role of these ToVs remains unclear and epidemiological studies is needed to study the causal relationship between virus presence and disease. In addition, the present review highlights the features and classification of the ToV, its host range, clinical signs, diagnosis, and suitable prevention and control measures to be implemented.

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