PORCINE TOROVIRUS

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SUMMARY

Etiology

- Porcine torovirus (ToV) is an enveloped RNA virus in the family *Coronaviridae*.
- There are four known species: porcine ToV, bovine ToV, equine ToV, and human ToV. Porcine ToV is thought to be more closely related to bovine ToV than equine ToV.
- The only porcine ToV strain isolated in the United States (PToV-NPL/2013) is 92% identical to the Chinese isolate PToV-SH1.

Cleaning and Disinfection

- Experimental data suggests that equine ToV is more easily heat-inactivated than some coronaviruses, and bovine ToV in fecal samples loses its infectivity within one to two days at temperatures above 4°C.
- Specific information on the disinfection of porcine ToV is lacking. Bovine ToV may lose infectivity upon treatment with chloroform or diethyl ether. ANIGENE HLD_4V, a disinfectant sold by MEDIMARK Scientific in the United Kingdom, is labeled as effective against porcine ToV.

Epidemiology

- In addition to pigs, bovines, equines, and humans, toroviruses or torovirus-like particles have also been detected in turkeys, goats, sheep, rodents, lagomorphs, and domestic cats.
- The zoonotic potential of porcine ToV is unclear. Early serological studies of farmworkers and veterinarians have not revealed a detectable antibody response against bovine and equine toroviruses.
- Porcine ToV seems to be widespread in the global swine population. The prevalence of porcine ToV in the United States is unknown.

Transmission

- Transmission of porcine ToV is thought to be fecal-oral. Rapid spread of porcine ToV between facilities seems to coincide with the practice of moving and regrouping weaned pigs.
- Adults may be chronically infected, acting as reservoir hosts.

Infection in Swine/Pathogenesis

- Infection with porcine ToV is generally subclinical; however, associated signs have included diarrhea, anorexia, dehydration, lethargy, depression, and potentially neurological or respiratory signs in other species.

Diagnosis

- Porcine ToV is not cultivatable.
- Immuno-electron microscopy (IEM) has been used to detect antibody-aggregated particles in feces and differentiate ToV from other coronaviruses.
- Conventional and real-time reverse transcription polymerase chain reaction (RT-PCR) assays have been developed.
- Indirect ELISA has also been used to detect antibodies against equine and porcine ToVs.

Immunity

- There is no vaccine for porcine ToV.

Prevention and Control

- There is no specific information available for control of porcine ToV.
- Common swine industry biosecurity practices should be in place.

Gaps in Preparedness

- Little is known about porcine ToV, including its pathogenicity and biological characteristics of the disease. Until the virus can be grown in culture, epidemiological studies can help increase understanding of the distribution and significance of infection.

OVERVIEW

The role of porcine torovirus (ToV) in enteric disease is unclear. The virus has been isolated from fecal samples and rectal swabs of piglets with diarrhea and is often found in conjunction with known enteric pathogens. There is no specific data available on associated morbidity and mortality in swine infected with porcine ToV. There are four distinct species of ToV – porcine, equine, bovine, and human – though turkeys, goats, rodents, rabbits, and cats may also be affected. Porcine ToV has a wide geographic range, with confirmed cases in Europe, China, Korea, South Africa, Canada, and recently the United States. Seroprevalence is high in swine herds of endemic areas, and the virus is thought to be transmitted pig-to-pig by fecal-oral contact. Infected piglets develop antibodies to the virus at a young age, following decline in the level of maternal antibodies. Adult swine are potential reservoir hosts, continuing to shed the virus and possibly contributing to its extensive spread among infected populations.

Genomes of several porcine ToV strains have been sequenced. In the United States, only one isolate has been identified by a Minnesota lab in a single pig that was also infected with porcine epidemic diarrhea virus (PEDV). However, bovine ToV was first discovered in the United States in 1979, suggesting that porcine ToV could already be circulating here undetected. The presence of chimeric hemagglutinin-esterase genes in some strains is indicative of the occurrence of recombination events in certain variants of porcine ToV. Existence of additional unidentified ToVs is a possibility, contributing to the potential for further adaptations of the viral genome.

A major limitation in our understanding of porcine ToV is a result of the inability to grow the virus in cell culture. The hemagglutinin-esterase protein of the viral envelope is a potential inhibitor of attempts to culture the virus *in vitro*, and the only culture-adapted ToV strains lack a functional version of this protein. Little is known about the survival of porcine ToV in the environment, and specific disinfection methods have not been described. Current diagnostic methods used in other countries include immune-electron microscopy, conventional and real-time reverse transcription polymerase chain reaction (RT-PCR), virus neutralization using culture-adapted equine ToV, Western blot assay, and enzyme-linked immunosorbent assay (ELISA). No vaccines are available for porcine ToV, and attempts to vaccinate piglets may be complicated by the presence of maternal antibodies.

Toroviruses are potentially zoonotic, though specific mechanisms of transmission are still unknown. The existence of human ToV or ToV-like particles, as well as the emergence in humans of severe acute respiratory syndrome (SARS), another member of the Coronaviridae family, calls for further investigation and vigilance. Among swine herds, continued spread of the virus appears to be facilitated by the movement and regrouping of piglets at times when they are highly susceptible to infection. Longitudinal studies have shown a significant increase in IgG antibodies at seven and eleven weeks, corresponding to movement of piglets to new facilities following weaning around three weeks of age and again after transport to finishing facilities at eight weeks. This should be carefully considered if pathogenicity of porcine ToV is established in the future.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Porcine torovirus (ToV) is an enveloped, single-stranded RNA virus belonging to the genus *Torovirus*, in the family *Coronaviridae*, subfamily *Torovirinae*.[1]

1.2 Strain Variability

Four species of ToV, based on their predominant host species, are currently recognized by the International Committee on Taxonomy of Viruses: porcine ToV, bovine ToV, equine ToV, and human ToV.[1] Bovine ToV has also been recognized as Breda virus (BRV), and equine ToV is also known as Berne virus (BEV).[10]

In 2014, a Minnesota diagnostic lab identified a porcine ToV isolate in the Unites States for the first time. Fecal/vomit swabs from the single pig were also positive for porcine epidemic diarrhea virus (PEDV). The complete genome of the isolate (PToV-NPL/2013) was found to be 92% identical to PToV-SH1, a Chinese isolate thought to be the first sequenced porcine ToV genome. The greatest viral protein diversity was found in the hemagglutinin-esterase (*HE*) gene, when comparing PToV-NPL/2013 to other known porcine ToV *HE* isolates. There was also variation in the gene for the spike (S) protein present on the viral envelope, which may affect virus interaction with a host.[4]

Porcine ToV is thought to be more closely related to bovine than equine ToV, and the sequencing of PToV-SH1 revealed a 79% shared identity with a strain of bovine ToV (Breda1). The greatest genetic divergence between porcine, equine, and bovine ToV appeared to be in the nonstructural protein NSP1, where frequent amino acid deletions and mutations occur. Variation also exists in the HE protein between porcine, bovine, and equine ToV, and this may play a role in the differing levels of virulence among the species.[5]

2. Cleaning and Disinfection

2.1 Survival

Survival of porcine ToV in the environment has not been reported. Enveloped viruses have a tendency to survive better at lower levels of humidity and air temperature,[6] and are generally unstable outside of the host.[7] Experimental data suggests that equine ToV is more easily heat-inactivated than some coronaviruses, and bovine ToV in fecal samples loses its infectivity within one to two days at temperatures above 4°C.[8]

2.2 Disinfection

Bovine ToV may lose infectivity upon treatment with chloroform or diethyl ether,[8] but no information specific to porcine ToV is currently available. The related coronavirus species are more susceptible to microbicides than non-enveloped viruses, and some can be inactivated by various concentrations and/or combinations of ethanol, isopropanol, iodine, sodium hypochlorite, phenolic compounds, and formaldehyde.[6] ANIGENE HLD_4V, a disinfectant sold by MEDIMARK Scientific, is labeled as effective against porcine ToV.[9] More research is needed on specific disinfection protocols for porcine ToV.

3. Epidemiology

3.1 Species Affected

In addition to porcine ToV, bovine ToV, equine ToV, and human ToV, toroviruses or torovirus-like particles have also been detected in turkeys,[3] goats, sheep, rodents, lagomorphs, and domestic cats.[10]

3.2 Zoonotic Potential

Pathogenicity and zoonotic potential of porcine ToV is not well understood. Early serological studies of farmworkers and veterinarians with high levels of exposure to animals revealed no detectable antibody to BRV or BEV.[7] However, some variants of porcine ToV contain *HE* genes, suggesting the possibility of recombination events and existence of yet unidentified ToVs. Changes in the viral genome and the presence of multiple strains necessitate continued surveillance and phylogenetic review of ToV.[11-13]

3.3 Geographic Distribution

Porcine ToV was first identified in piglets in the Netherlands in 1998 and has since been discovered in pigs from several other European countries including Belgium, Switzerland, Hungary, Spain, and Italy, as well as Korea[11] and a single pig in the United States.[4] Porcine ToV has also been reported in China, the UK,[3] Canada, and South Africa.[10] Existing epidemiological data suggests that porcine ToV may be prevalent and widespread in global swine populations, despite its occurrence at primarily subclinical levels.[3]

3.4 Morbidity and Mortality

Limited epidemiological studies indicate that swine herds generally exhibit high seroprevalence in endemic countries, though pathogenicity remains unclear.[2,3] To date, the prevalence of porcine ToV in the United States remains unknown.

4. Transmission

Transmission of ToV is thought to occur by fecal-oral contact, though an early isolate, described as bovine respiratory ToV (BRTV) from calf respiratory tissue, suggests potential for infection by aerosolized virus inhalation.[7] Rapid spread of porcine ToV between facilities seems to coincide with the practice of moving and regrouping weaned piglets around the time of greatest susceptibility, when maternal antibodies have waned and the piglets have not yet mounted their own antibody response.[14] Chronically infected adult swine may act as reservoir hosts, continuously shedding the virus, though molecular detection is needed to confirm this hypothesis.[2] Whatever the true mode of transmission, the high seroprevalence reported in Spain is indicative of the endemic nature of the virus in some swine populations.[2]

5. Infection in Swine/Pathogenesis

5.1 Clinical Signs

Pigs can shed the virus but generally show no clinical signs. ToV is thought to cause diarrhea, anorexia, dehydration, lethargy, depression, and potentially neurological or respiratory signs in other species.[10]

5.2 Postmortem Lesions

The pathogenicity of porcine ToV remains unclear, and there is no reported link between gut lesions and infection with the virus.[15]

6. Diagnosis

6.1 Clinical History

ToV has been associated with diarrhea in piglets, although a direct link between the virus and enteric disease in swine has not been clearly established.[15] Epidemiological studies suggest that there is not a direct correlation between seroprevalence of porcine ToV antibody and a farm's breeding system,

biosecurity measures, or clinical history of diarrhea in the pigs.[2] However, other studies have been able to detect porcine ToV in pigs with diarrhea in the absence of simultaneous infection with other known enteric pathogens, including PEDV, porcine group A-C rotaviruses (PRV A-C), transmissible gastroenteritis virus (TGEV), astrovirus (AV), mammalian orthoreovirus (MRV), porcine sapovirus (PSaV), porcine norovirus (PNoV), and porcine kobuvirus (PKBV).[3,16] While this does not definitively establish pathogenicity, it is an area for further study.

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Techniques for cell culture of porcine ToV have not been described. The *HE* protein of the virus, while present in field strains and thought to be important to *in vivo* infection, may play an inhibitory role in attempts to culture the virus *in vitro*.[17] Immuno-electron microscopy (IEM) has been used to detect antibody-aggregated particles in feces and differentiate ToV from other coronaviruses. Conventional and real-time reverse RT-PCR assays have also been developed.[15] More specifically, a one-step SYBR Green real-time RT-PCR has shown potential for more rapid, specific, and sensitive detection of porcine ToV.[14,18] It is important to note that RT-PCR alone may not be an accurate measure to monitor prevalence, due to the initiation of an immune response that may effectively cure affected individuals.[3]

6.3 Tests to Detect Antibody

An indirect ELISA using a recombinant purified porcine ToV N protein antigen has been developed, and correlated well with results of a virus neutralization (VN) assay using a culture-adapted equine ToV[15,19] and a Western blot assay also detecting IgG antibodies against the N protein.[19] Similarly, indirect ELISA has been used to detect antibodies against two distinctly identified lineages of porcine ToV HE proteins.[17]

6.4 Samples

6.4.1 Preferred Samples

Fecal samples are appropriate for IEM,[15] while both rectal swabs[14] and fecal samples have been used for RT-PCR assays.[15] Intestinal mucosa and contents have also been utilized post-mortem for RT-PCR.[16]

6.4.2 Oral Fluids

The use of oral fluids as a diagnostic specimen has not been evaluated for porcine ToV.

7. Immunity

7. 1Post-Exposure

Maternal antibodies provide some protection to exposed piglets up until the age of weaning (three weeks), and infected piglets will have produced detectable levels of IgG antibodies against ToV by around five weeks of age. A large scale epidemiologic study of 100 farms in ten regions in Spain found that >99% of pigs over 11 weeks of age were seropositive for anti-porcine ToV IgG, suggesting a continuous spread of the virus on farms. Antibodies against porcine ToV were detected on every farm in the study.[2]

There is discussion of the continued reinfection of intestinal cells despite the presence of serum IgG, suggesting a potential gap in protection at mucosal surfaces. Reports of ToV infection of M cells further support this theory.[14]

7.2 Vaccines

Presently, there are no vaccines available for porcine ToV.[10]

7.3 Cross-protection

Equine, bovine, and porcine ToV are serologically related as evidenced by cross-reactive antibody binding. Equine ToV is neutralized by antibody present in the serum of bovine ToV infected and porcine ToV infected animals.[10]

8. Prevention and Control

Specific information on control of porcine ToV is not available; however, common biosecurity practices should be followed when handling pigs. Care should be taken to avoid spreading fecal matter or soiled bedding to areas where it could infect other animals. Use of available diagnostics and increased surveillance of herds may aid in controlling the spread of the virus.[10]

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

Porcine ToV is not covered in the 2015 OIE Terrestrial Animal Health Code and there are no recommendations on importation of swine or pork.

10. Gaps in Preparedness

The inability to grow porcine ToV in cell culture greatly limits our current understanding of the pathogenicity and biological characteristics of the disease.[11] Until that is achieved, the clinical significance of ToV could be better understood with detailed epidemiological surveys of large populations, longitudinal cohort studies, and animal challenge studies. If and when pathogenicity can be clearly established, the development of a suitable vaccine could help to protect both swine and human populations.[10]

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