PORCINE BOCAVIRUS

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SUMMARY

Etiology

- Porcine bocavirus (PBoV) is a non-enveloped single stranded DNA virus in the family *Parvoviridae*, genus *Bocavirus*.
- Although bocaviruses (BoVs) have been recognized in veterinary medicine since the early 1960s, PBoV was not isolated until 2009 in Sweden, where the virus was found in the lymph nodes of pigs with post-weaning multisystemic wasting syndrome (PMWS).
- Currently there are seven known PBoV genotypes, divided into three distinct groups based on the sequence of the VP1 gene: PBoV G1, PBoV G2, and PBoV G3.

Cleaning and Disinfection

- Survival of PBoV in the environment has not been reported.
- In general, parvoviruses are very resistant to dry heat, requiring 70°C (158°F) for ten minutes to become inactivated. Although no specific information is available for PBoV, parvoviruses are often resistant to common disinfectants.

Epidemiology

- In addition to infecting pigs, BoVs have been linked to respiratory disease, gastrointestinal disease, and reproductive loss in cattle. Ape-variant BoV has been detected in Western gorillas with enteritis, and serological evidence of infection has been detected in chimpanzees and gorillas in Cameroon. Sea lions have tested positive for BoV, and canine and feline BoVs have been detected in tissue samples and feces, most recently from stray dogs and cats in Hong Kong.
- No cases of PBoV have been reported in humans; however, PBoVs and human strains are antigenically cross-reactive. Viral recombination has also been documented in both human and porcine BoVs, raising the possibility of cross-species transmission.
- PBoVs have been identified in North America, Asia, the UK, Eastern Europe, and Africa.
- Reported PBoV prevalence in sick pigs in China is around 11%, while U.S. studies have documented prevalence rates in sick pigs of approximately 43% and 59%. In China, PBoV infection is most common in the spring (March–May). Infection occurs most commonly in weaned piglets.

Transmission

- Transmission of PBoV remains unclear. Viral DNA can be detected in a range of tissues, including lymph nodes, serum, lung, oral fluids, and feces.

Infection in Swine/Pathogenesis

- The pathogenesis of PBoV and its ability to induce clinical signs is not well understood. Viral co-infections are common in PBoV-positive pigs. To date, PBoVs have been reported in pigs with PMWS, respiratory disease, diarrhea, and in asymptomatic swine. Reproductive failure may also occur. A single case of PBoV-associated encephalitis has been reported. It does not appear that any studies of experimental PBoV infection have been conducted in pigs.

Diagnosis

- Clinical specimens to submit for PBoV testing include serum, lung, lymph nodes, tonsil, liver, nasopharyngeal swabs, and feces. The virus has also been detected in oral fluids.
- PBoV has been successfully propagated in primary pig kidney cells and identified via electron microscopy and immunofluorescence assays.
- Sequence-based methods to identify PBoV include polymerase chain reaction (PCR), TaqMan-based quantitative PCR (qPCR), multiplex qPCR, loop-mediated isothermal amplification, nanoPCR, duplex nanoPCR (PBoV and pseudorabies virus), and sequence-independent single primer amplification (SISPA).
- Monoclonal antibodies have been developed for use in an antigen-detecting enzyme linked immunosorbent assay (ELISA) and an ELISA targeted anti-PBoV IgG has recently been described.

Immunity

- There is evidence that maternal antibodies might be involved in protecting young piglets from infection.
- There is no vaccine for PBoV.

Prevention and Control

- There is no specific information available for control of PBoV.
- Common swine industry biosecurity practices should be in place at all swine production sites.

Gaps in Preparedness

- Little is known about PBoV, including its pathogenicity and modes of transmission. More research should be done in order to understand the significance of infection and how or whether it may be prevented.

OVERVIEW

Porcine bocaviruses (PBoVs) are non-enveloped, single stranded DNA viruses belonging to the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Bocavirus*. Although bocaviruses (BoVs) have been recognized in veterinary medicine since the early 1960s, PBoV was not isolated until 2009 in Sweden, where the virus was found in the lymph nodes of pigs with post-weaning multisystemic wasting syndrome (PMWS). Currently there are seven known PBoV genotypes, divided into three distinct groups based on sequencing of the VP1 gene, which encodes for a capsid protein. Porcine parvovirus (PPV) is distantly related to PBoV. The survival of PBoVs in the environment has not been reported. There is no information on specific disinfection protocols for PBoV. However, parvoviruses such as PPV generally have a high resistance to dry heat and many disinfectants and tend to survive in the environment relatively well.

BoVs have been described in humans and several animal species. Bovine bocavirus (BPV) is linked to diarrhea and respiratory disease in cattle, as well as reproductive loss. Ape BoV variants have been detected in the feces of captive Western gorillas with enteritis, and serological studies have detected antibodies to BoV in wild chimpanzees and gorillas in Cameroon. Sea lions have tested positive for BoV, and canine and feline BoVs have also been described, most recently in samples from stray dogs and cats collected in Hong Kong. There are at least four BoVs that affect humans (HBoV), and PBoV shares greater homology with HBoV than other animal strains. To date, there have been no reported cases of PBoV in humans. However, recombination events have been documented in both human and porcine BoVs, and the possibility of transmission across species should be considered.

PBoV was first identified in the lymph nodes of pigs with PMWS in Sweden in 2009. Since this discovery, PBoVs have been identified in North America, Asia, the UK, Eastern Europe, and Africa. Serological studies of PBoV are limited but the virus seems to be common in some swine herds. Overall PBoV prevalence in sick pigs in China has been reported at around 11%, while studies in the U.S. have documented overall prevalence in sick pigs of 43% and 59%. In China, the highest incidence of disease seems to occur in the spring, from March–May. Infection is most common in weaned pigs, where morbidity of 50–100% and mortality of 20–60% have been observed. Viral co-infections are common however, and the role of PBoV as a primary pathogen remains poorly characterized.

Transmission of PBoV is also unclear. Viral DNA can be detected in lymph nodes, serum, lung, oral fluids and feces. Asymptomatic infections are common. Clinically affected pigs may show gastrointestinal and respiratory signs, as well as reproductive failure. A case of PBoV-linked encephalitis has also been reported. It does not appear that any studies of experimental PBoV infection have been conducted in pigs.

Clinical specimens to submit for PBoV testing include serum, lung, lymph nodes, tonsil, liver, nasopharyngeal swabs, and feces. The virus has also been detected in oral fluids. PBoV has been successfully propagated in primary pig kidney cells and identified via electron microscopy and immunofluorescence assays. Sequence-based methods to identify PBoV include polymerase chain reaction (PCR), TaqMan-based quantitative PCR (qPCR), multiplex qPCR, loop-mediated isothermal amplification, nanoPCR, duplex nanoPCR (PBoV and pseudorabies virus), and sequence-independent single primer amplification (SISPA). Monoclonal antibodies have been developed for use in an antigen-detecting enzyme linked immunosorbent assay (ELISA) and an ELISA targeted anti-PBoV IgG has recently been described.

There are currently no vaccines available for PBoV. Specific information on prevention and control of PBoV is not available. Common swine industry biosecurity practices should be in place at all swine production sites.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Bocaviruses (BoVs) belong to the family *Parvoviridae*, subfamily *Parvovirinae*. The genus *Bocavirus* is named after its first two members, **bo**vine parvovirus (BPV) and minute virus of **ca**nines (MVC). BoVs are small, non-enveloped viruses with icosahedral symmetry encoded by a single-stranded linear DNA genome.[1] Although BoVs have been recognized in veterinary medicine since the early 1960s, porcine BoV (PBoV) was not isolated until 2009 in Sweden, where the virus was found in the lymph nodes of pigs with post-weaning multisystemic wasting syndrome (PMWS).[2-4]

1.2 Strain Variability

BoVs are distinct from the other members of the subfamily *Parvovirinae* as they contain three major open reading frames (ORFs), coding for four genes (NS1, NP1, VP1/2).[5] In other parvoviruses, NS1 is essential for viral DNA replication, and NP1 is suspected to play a role as well.[6] In human isolates, NP1 is known to block IFN production[6] and early studies indicate that it has a similar function in PBoV.[7,8] VP1/2 encode for capsid proteins. Porcine parvovirus (PPV) is a member of the subfamily *Parvovirinae* and is distantly related to PBoV[9], as is porcine hokovirus (PHoV), a novel porcine parvovirus first described in 2008.[10]

The first BoV isolated from pigs, PBo-likeV, was identified in Sweden in 2009. In lymph nodes from pigs with PMWS, an 1879-bp sequence characterizing the NP1 and VP1/2 genes was described.[3] Soon after, PBoV was isolated from pigs with respiratory disease in China. Compared to Swedish strains, the VP1/2 genes of Chinese strains were highly conserved.[11] Further studies from China in 2010 identified two novel isolates (PBoV1/2-CHN) via NS1 gene sequencing using fecal samples from healthy pigs.[12] In the same study, partial sequences of two additional boca-like viruses (6V and 7V) were also described.[12] PBoV1 and PBoV2 strains were most prevalent in China from 2006–2011.[13]

PBoV3/4-UK were identified from small intestinal and fecal samples from pigs with PMWS in Northern Ireland in 2011.[14] Similar strains were described in China (PBoV3/4-HK), again based on NS1 and VP1 sequencing of virus in tissue samples from healthy, sick, and dead pigs.[15] From approximately 2010–2012, PBoV3 subtypes were epidemic in both China and the U.S.[13] A further related virus, PBoV3C, was isolated from the feces of healthy piglets in China in 2012.[16] PBoV5 was detected in 2012, in fecal samples from piglets with clinical diarrhea on one Chinese farm.[17] A novel strain described in China in 2014, swBoV CH437, was recently identified in fecal samples from healthy pigs.[18] A 2014 study of PBoV in sick pigs in the U.S. identified 18 partial or complete genomes, six of which were novel. In particular, the VP2 gene of PBoV G3 isolates showed high diversity.[19] Currently, PBoV3C, PBoV5, and PBoV3/4 strains seem to be most prevalent in China and the U.S.[13]

New PBoVs will likely continue to emerge. Simultaneous infection with more than one PBoV is possible and recombination events between PBoVs have been documented in several instances [15,16,19] For example, an isolate provisionally named PBoV-KU14 was detected in 2015, in domestic pigs with respiratory disease from South Korea. This virus, with a NP1 gene truncation, was deemed likely to be the result of crossover recombination.[20]

Known PBoVs have been classified into seven genotypes[6] based mostly on the sequence of VP1, a capsid protein likely to influence tissue tropism.[21] Subgroups have been defined based on phylogenetic clustering and the homology matrix of known PBoVs. Recognized PBoV subgroups include the following:

- PBoV G1, which includes PBo-likeV, PBoV-SX and PBoV1-H18;
- PBoV G2, which includes PBoV1/2-CHN and PBoV2-A6; and
- PBoV G3, which includes PBoV3/4-UK, PBoV3/4-HK and PBoV3C.[16]

2. Cleaning and Disinfection

2.1 Survival

Survival of PBoV in the environment has not been reported. Parvoviruses generally have a high resistance to dry heat; exposure to 70°C (158°F) for ten minutes is required for inactivation.[22] Parvoviruses are generally stable from pH 3–9.[23]

2.2 Disinfection

Parvoviruses are resistant to disinfection; however, there is no information specific to PBoV currently available. Parvoviruses can be inactivated by treatment with formalin, β-propriolactone, hydroxylamine, and ultraviolet light.[23] Oxidizing agents (e.g., sodium hypochlorite) may be effective at high concentrations. PPV, a related virus, is resistant to inactivation by ethanol (70%) and quaternary ammonium (0.05%), as well as low concentrations of sodium hypochlorite (2500ppm) and peracetic acid (0.2%). PPV is readily inactivated by aldehyde-based disinfectants and higher concentrations of sodium hypochlorite (25,000ppm) and hydrogen peroxide (7.5%).[24]

3. Epidemiology

3.1 Species Affected

BoVs have been implicated in both respiratory and gastrointestinal disease in animals, as well as reproductive loss. In addition to domestic[3,11,12,14-18] and wild[25,26] pigs, BoVs have been detected in other animal species.

- The first BoV was described in the early 1960s, when a hemadsorbing virus, now known to be a bovine bocavirus (BPV), was identified in the feces of diarrheic calves in the U.S.[27] Three serologically distinct hemadsorbing isolates were described in Japanese cattle with respiratory disease and diarrhea in 1966[28] and further characterized as parvoviruses in the 1970s.[29] The virus has also been associated with reproductive failure.[30]
- A 2010 report identified a novel BoV in the feces of captive Western gorillas with acute enteritis living in North America.[31] A further study of wild apes showed that chimpanzees and gorillas tested in Cameroon were seropositive for human bocavirus (HBoV). Ape HBoV variants have also been identified in fecal samples.[32]
- In 2011, a BoV was detected in the feces of wild and temporarily captive California sea lions.[33]
- Canine BoV (CBoV) has been isolated from dogs with respiratory disease.[34] A 2012 report identified CBoV in samples from stray dogs in Hong Kong including feces, urine, blood, and nasal swabs.[35] In the same study, a feline bocavirus (FBoV) was described for the first time in similar samples collected from stray cats in Hong Kong.[35]

3.2 Zoonotic Potential

At least four different HBoV species have been described, linked to both respiratory and gastrointestinal disease.[36] While animal BoVs are relatively distinct from human strains, PBoV appears to share greater genetic homology with HBoV than other animal strains based on the NS1 gene.[5] There have been no reported cases of PBoV in humans. However, recombination events have been documented in both human and porcine BoVs, and the possibility of transmission across species should be considered.[6]

3.3 Geographic Distribution

PBoV was first identified in the lymph nodes of pigs with PMWS in Sweden in 2009.[3] Since this discovery, PBoVs have been identified in Sweden, China, the U.S., Canada, Mexico, Romania, Hungary, Uganda, Korea, Cameroon, the UK[6] and Ireland.[5,14] In the U.S., it has been suggested that Minnesota and North Carolina were the geographical origins of PBoV.[13]

3.4 Morbidity and Mortality

Overall prevalence of PBoV in sick pigs (e.g., those with respiratory, enteric, and/or reproductive disease) in China has been reported as 11.4%.[37] Several Chinese studies have reported the prevalence of specific PBoV strains, including 21.9% (PBoV G2)[21], 30.5% (PBoV G1)[21], 38.3% (6V/7V)[21], 38.7% (PBoV G1)[11], and 56.1% (PBoV G1).[38] In healthy pigs, PBoV prevalence in China ranges from 7.3[11]– 64.4%.[1] In the U.S., an overall PBoV prevalence of 58.7% has been documented in sick pigs (samples tested included lung, lymph node, serum, and feces from pigs with various disease conditions).[19] Another report found that 43.3% of U.S. pigs sampled with respiratory illness or diarrhea were positive for PBoV.[39] Serological testing has found that over 90% of pigs and nearly 60% of wild boars in Japan are positive for PBoV.[26]

Viral co-infections seem to be common in PBoV-positive pigs. Early evidence in Sweden associated PBoV infection with PMWS[3] and other studies in China[38], Sweden[40], Northern Ireland[14], the Czech Republic[41], and the UK[42] have supported these findings. For example, one study showed that 88% of PMWS-positive piglets were also positive for PBoV, while only 46% of healthy piglets tested positive for the virus.[40] PBoV infection is also associated with respiratory and gastrointestinal disease, as well as reproductive loss. One study demonstrated a much higher frequency of PBo-likeV infections in pigs with respiratory disease and reproductive failure (39%) compared to healthy pigs (7%).[11] Another U.S. study found that nearly 49% of pigs with diarrhea and 30% of pigs with respiratory disease were positive for PBoV.[39] However, PBoV-positive pigs with respiratory disease were concurrently infected with other pathogens including rotavirus, hepatitis E virus (HEV) and porcine reproductive and respiratory syndrome virus (PRRSV). Of the PBoV-positive pigs with diarrhea, 57% also tested positive for porcine circovirus type 2 (PCV2), porcine astrovirus, or porcine rotavirus.[39] Other pathogens associated with PBoV infection include porcine torque teno virus (PTTV), classical swine fever virus (CSFV), porcine epidemic diarrhea virus (PEDV), porcine kobuvirus (PKoV), and transmissible gastroenteritis virus (TGEV).

The prevalence of PBoV infection varies with season and age. In China, one study indicated that the prevalence of PBoV infection was highest in spring (March–May) compared to summer, autumn, or winter.[11] PBo-likeV infection seems to be more prevalent in weaned piglets than in other age groups in both domestic and wild swine populations.[11,25] In a study of samples from diseased pigs (with respiratory and/or reproductive signs), the highest morbidity (50–100%) and mortality (20–60%) were observed in pigs 15–70 days old. In sows greater than one year-of-age and boars greater than two years-of-age, few deaths occurred.[11] The low prevalence of PBoV infection in very young piglets suggests a passage of protective maternal immunity.[11,25] Though testing has been limited, the absence of recorded infection in aborted fetuses suggests that the virus does not cross the placenta.[11]

4. Transmission

Transmission of PBoV remains unclear. Viral DNA can be detected in a range of tissues, including lymph nodes, serum, lung, oral fluids and feces.[5] In China, PBoV genomes have been found in slaughter offal (e.g., blood, lymph nodes, feces, etc.).[37]

5. Infection in Swine/Pathogenesis

The pathogenesis of PBoV infection is not well understood. PBoV has been found in pigs co-infected with other viral diseases of swine.[7] Pigs can also be concurrently infected with more than one PBoV.[15,19]

5.1 Clinical Signs

PBoV has been isolated from asymptomatic swine. In addition, the high likelihood of co-infection with other viruses makes it difficult to evaluate signs caused by PBoV.[5] Studies suggest that infected pigs may show respiratory symptoms (e.g., high fever, acute dyspnea, panting) and reproductive failure (e.g.,

abortion/stillbirth for sows, low semen quality for boars).[11] Gastrointestinal disease is also associated with PBoV.[17] In 2013, a PBoV-infected 6-week-old piglet in Germany presented with coughing, growth retardation, and diarrhea, and was diagnosed with encephalitis postmortem.[43] BoVs in other species, such as BPV and CMV also cause disease of the gastrointestinal and respiratory tracts, as well as reproductive disorders.[2]

5.2 Postmortem Lesions

Tissue samples collected from naturally infected PBoV-positive pigs in China were examined, and pathology showed severe lesions of the viscera including enlarged lymph nodes, blood spots on the lungs and liver, and infarcts in the spleen and kidney.[11] However, viral co-infections including PCV2, PTTV, and CSFV were also documented in many samples, making interpretation of observed lesions difficult.

Histology of a PBoV-infected 6-week-old piglet from Germany revealed interstitial pneumonia; a mild, multifocal, lymphohistiocytic panencephalitis that affected the cerebrum and cerebellum, including brain stem and medulla oblongata; and a mild, multifocal, lymphohistiocytic panmyelitis. The only viral sequence detected was PBoV. *Mycoplasma hyorhinis* was detected via multiplex PCR within the lung and pulmonary lymph nodes.[43]

6. Diagnosis

6.1 Clinical History

PBoV has been found in pigs with PMWS, diarrhea, respiratory disease, encephalomyelitis, generalized illness (inappetence, lethargy, fever, wasting), and apparently healthy pigs.[6,11,43,44]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Novel PBoVs have been identified based on amplification and partial (targeting the NS1, NP1, and VP1/2 genes) or full genome sequencing as detailed in section 1.2.

To date, PBoV has been successfully propagated in primary pig kidney cells.[14,45] PBoV can be identified via electron microscopy and immunofluorescence assays.[14] Sequence-based methods to identify PBoV include polymerase chain reaction (PCR)[11], TaqMan-based quantitative PCR (qPCR)[38], multiplex qPCR[19,46], loop-mediated isothermal amplification[47], nanoPCR[48], and duplex nanoPCR (PBoV and pseudorabies virus).[49] High-throughput[16] and next generation sequencing[43] methods have been described. Sequence-independent single primer amplification (SISPA), a primer-initiated technique by which nucleic acids of an unknown sequence can be amplified with sequence-independent PCR methods using a single primer, has been used to identify PBoV.[12] Monoclonal antibodies have been developed for use in an antigen-detecting enzyme linked immunosorbent assay (ELISA).[50]

6.3 Tests to Detect Antibody

An ELISA detecting anti-PBoV IgG has recently been described.[26]

6.4 Samples

6.4.1 Preferred Samples

Clinical specimens to submit for PBoV testing include serum, lung, lymph nodes, tonsil, liver, nasopharyngeal swabs, and feces.[6] The detection rate in nasopharyngeal swabs has been found to be significantly higher in dead pigs than in healthy pigs.[15]

6.4.2 Oral Fluids

PBoV has been detected in oral fluids.[44]

7. Immunity

7.1 Post-exposure

Serological studies of PBoV in pigs are limited. The low prevalence of PBoV in very young piglets suggests protective maternal immunity.[11,25] There is speculation that PBoV might be an immunosuppressive pathogen, as the non-structural protein NP1 has been shown to suppress IFN production.[7,8]

7.2 Vaccines

Presently, there are no vaccines available for PBoV or BoVs found in other species.

7.3 Cross-protection

According to phylogenetic analyses, PBoV has a close relationship with other BoVs such as FBoV and BPV.[6] Experimentally, PBoV-like particles (PBoV-LPs) generated by a recombinant baculovirus were not cross-reactive with PCV2 but were cross-reactive with HBoV 1, 2, 3 and 4.[26] Pigs can be simultaneously infected with more than one PBoV.[19]

8. Prevention and Control

Specific information on control of PBoV is not available; however, common biosecurity practices should be followed when handling pigs. Care should be taken to avoid spreading feces or soiled bedding to areas where it could infect other animals.

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

PBoV is not covered in 2016 OIE Terrestrial Animal Health Code and there are no recommendations on importation of swine or pork.

10. Gaps in Preparedness

PBoV has been isolated from pigs with PMWS, respiratory disease, diarrhea, reproductive disease, and encephalomyelitis, as well as heathy pigs. However, little is known about the clinical importance of PBoV. Viral co-infections are common in PBoV-positive pigs. Further information is needed about the pathogenesis of this virus. No vaccines are currently available or in development. To prevent infection with PBoV, more information is needed on virus survival in the environment and susceptibility to disinfection.

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