PORCINE KOBUVIRUS

Prepared for the Swine Health Information Center
By the Center for Food Security and Public Health,
College of Veterinary Medicine,
Iowa State University
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SUMMARY

Etiology

- Porcine kobuvirus (PKoV) is a small, non-enveloped RNA virus in the family *Picornaviridae*.
- There are three distinct clusters within the genus *Kobuvirus*: Aichivirus A (AiV-A) includes human AiV-1, canine KoV-1, and murine KoV-1. Aichivirus B (AiV-B) includes bovine KoV-1 and sheep KoV-1. Aichivirus C (AiV-C) includes porcine KoV-1 (PKoV/AiV-C).[1]

Cleaning and Disinfection

- AiV-1 is readily inactivated at 56ºC after 20 minutes.
- There is no published information about the susceptibility of PKoV/AiV-C to disinfectants. Kobuviruses (KoVs) are potentially susceptible to disinfection with acids like acetic acid, aldehydes like glutaraldehyde, alkalis like sodium hydroxide, and oxidizing agents like Virkon-S®[11].

Epidemiology

- Kobuviruses infect many different species. The AiV-C cluster contains swine viruses exclusively.
- PKoV/AiV-C has been isolated from swine herds in China, Thailand, Japan, South Korea, Italy, Hungary, Czech Republic, the United States, the Netherlands, Kenya, Uganda, and Brazil.
- Prevalence in domestic pigs ranges from 13–99%. One study of pigs in the United States showed that 21.7% of healthy and 21.9% of diarrheic samples were PKoV/AiV-C-positive.

Transmission

- Transmission is thought to be fecal-oral.
- Wild boars might be a source of infection for domestic swine.

Infection in Swine/Pathogenesis

- PKoV/AiV-C has been implicated as the cause of an outbreak of diarrhea, dehydration, and vomiting in Chinese piglets. It has been suggested that PKoV/AiV-C can only induce diarrhea in the presence of other pathogens like transmissible gastroenteritis virus or rotavirus.

Diagnosis

- AiV-B and PKoV/AiV-C have never been propagated *in vitro*.

- Reverse transcriptase polymerase chain reaction (RT-PCR) can be used to detect PKoV/AiV-C in both fecal and serum samples of pigs.
- Antigen capture enzyme linked immunosorbent assays (ELISA) and electron microscopy have successfully diagnosed KoV antigens.
- Human anti-AiV-A antibodies have been detected by serum neutralization. No studies have been conducted evaluating seroconversion to PKoV/AiV-C in pigs.

Immunity

- Piglets can be re-infected with the same or different PKoVs over time.
- There are no vaccines for any KoVs, including PKoV.

Prevention and Control

- Farm sanitation and quarantine of sick pigs may help decrease the transmission of KoVs.

Gaps in Preparedness

- The true role of PKoV/AiV-C as a pathogen remains unclear. Further research is needed on the pathogenesis of PKoV/AiV-C-induced disease.
- No vaccines are available or in development.
- Kobuviruses are common in the environment but their hardiness and susceptibility to disinfection requires additional investigation.

OVERVIEW

Porcine kobuvirus (PKoV) is a suspected cause of diarrhea in young piglets. Although PKoV has been found in feces from ill pigs, co-infection with other enteric viruses is common and may play a role in the clinical signs observed. A possible PKoV-related outbreak in piglets in China caused diarrhea, dehydration, vomiting, and death. PKoV was first isolated from Hungarian pigs in 2008 and has since been found worldwide.

PKoV belongs to the genus *Kobuvirus* in the family *Picornaviridae*. There are three subtypes within the genus *Kobuvirus* based on affected species: Aichivirus-A (AiV-A), AiV-B, and AiV-C. AIV-C is the subtype that affects swine. Viruses belonging to AiV-B and AiV-C have never been isolated successfully. AiV-A has been isolated and propagated in cell culture, but it takes up to six weeks. PKoV has been detected in both fecal and serum samples of pigs by reverse transcriptase polymerase chain reaction (RT-PCR) assays, the most reliable and commonly used diagnostic tool for PKoV. Antigen capture enzyme linked immunosorbent assays (ELISA), and electron microscopy have successfully diagnosed KoV antigens. Human anti-AiV-A antibodies have been detected by serum neutralization.

Vaccines for KoVs have not been developed. Little is known about the immune response induced by PKoV infection. However, it is thought that immunity is short-lived and is not protective against virus-induced diarrhea as reinfection of a single pig with multiple strains of PKoV has been documented.

KoVs present a health hazard for humans. KoVs has been isolated from shellfish, clams, oysters, and groundwater and are identified as a cause of foodborne illness. No zoonotic infections have been reported; however, cross-species transmission, co-infection with multiple PKoV strains, and viral recombination events have all been documented.

Further research is required to better understand the role of PKoV in clinical disease, its pathogenesis, and whether vaccine development is feasible to prevent and control outbreaks.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Kobuviruses (KoVs) are members of the family *Picornaviridae*, the order *Picornavirales*, and the genus *Kobuvirus*, one of 8 genera of the family. KoVs are small, non-enveloped, round, single-stranded positive-sense RNA virus with one large open reading frame encoding for a single polyprotein.[1] The name kobuvirus comes from the Japanese word *kobu*, meaning "bump," due to the morphological appearance of virus particles by electron microscopy.[1]

1.2 Strain Variability

Within the genus *Kobuvirus* there are three distinct clusters. Aichivirus A (AiV-A) includes human AiV-1, canine KoV-1, and murine KoV-1. Aichivirus B (AiV-B) includes bovine KoV-1 and sheep KoV-1. Aichivirus C (AiV-C) includes porcine KoV-1 (PKoV/AiV-C).[1]

PKoV/AiV-C was discovered separately in Hungary and China using RT-PCR to detect calicivirus in stool samples.[1] PCR fragments uncharacteristic of calicivirus were present and sequencing revealed the fragments to be similar to AiV-A and AiV-B, thereby placing the virus within the *Kobuvirus* genus. Further analysis revealed PKoV/AiV-C to be distantly related to both AiV-A and AiV-B, identifying it as a new species within the genus.[1]

KoVs have a single open reading frame that results in a polyprotein; it produces three structural and seven non-structural proteins.[1] The 3D gene, which encodes the RNA-dependent RNA polymerase, is the most conserved region of the genome.[5-7] VP1, a structural capsid protein region, is highly variable among strains and is important for genomic sequence analysis, strain differentiation, and strain identification.[8]

Classification as an AiV requires 70% amino acid homology within the full polyprotein, 70% P1 coding region, and 80% amino acid homology in the 2 and 3CD region.[1] The 3CD region of PKoV is similar to human and bovine KoVs; however, the virus is distinct enough to be classified separately.[1] Fecal samples from diarrheic and non-diarrheic pigs in Italy revealed a dichotomous clustering of PKoV. One clustered closely with Chinese and Korean strains (97% homology). The other clustered closely with a Kenyan strain (93% homology)[6].

Complete genome sequences for two Hungarian PKoV/AiV-C strains, including the prototype strain, and three Chinese PKoV/AiV-C strains are available in GenBank.[6]A sixth PKoV/AiV-C variant was recently sequenced and found to share approximately 88% identity with nucleotide sequences of the five strains currently known.[8]

2. Cleaning and Disinfection

2.1 Survival

AiV-1 is present in groundwater, sewage, river water, and shellfish.[10] Further studies are needed to evaluate the hardiness of PKoV/AiV-C in the environment.

2.2 Disinfection

AiV-1 is stable over a wide pH range (pH 2–10); it is resistant to alcohols (90% ethanol and isopropanol after five minutes) and insensitive to chlorine-treated water.[10] The virus is also resistant to chloroform treatment.[2]

AiV-1 is readily inactivated at 56ºC after 20 minutes.[10] Temperatures achieved by composting of dairy manure (between 55 and 70°C for at least for 3 days for a static aerated-pile system) are adequate for inactivation of AiV. KoVs are potentially susceptible to disinfection with acids like acetic acid, aldehydes like glutaraldehyde, alkalis like sodium hydroxide, and oxidizing agents like Virkon-S®[11]. No studies have specifically evaluated the stability of PKoV/AiV-C.

3. Epidemiology

3.1 Species Affected

Kobuviruses from the AiV-A cluster affect humans, canines, and goats. AiV-B viruses infect bovines and sheep. The AiV-C cluster contains swine viruses exclusively.[1] PKoV/AiV-C has been detected in wild boars in Hungary who had no contact with domestic pigs.[12]

3.2 Zoonotic Potential

The zoonotic potential of PKoV remains unclear and understudied. Cross-species transmission is suspected; in one case, a bovine-related KoV was isolated from a six-month-old piglet.[1] In pigs, co-infection with multiple distinct PKoV/AiV-C isolates has also been reported.[13]

AiV-1 was first isolated from the feces of human patients who fell ill after consuming oysters during winter months in Japan.[9] PKoV/AiV-C has never been isolated from human diarrhea cases.[3,13,14]

3.3 Geographic Distribution

PKoV/AiV-C is endemic in many countries and has been isolated worldwide. PKoV/AiV-C has been isolated from swine herds in China[15], Thailand[7], Japan[3], South Korea[16], Italy[6], Hungary[17], Czech Republic[16,] , United States[18], Netherlands[4], Kenya[5], Uganda[5], and Brazil[19].

3.4 Morbidity and Mortality

PKoV/AiV-C prevalence in domestic pigs ranges from 13–99%. Virus has been isolated from both healthy and diarrheic pigs.[3,5,7,13,20] In the United States, 21.7% of healthy and 21.9% of diarrheic samples were PKoV/AiV-C-positive.[18] In China, 82.4% of districts reported presence of PKoV/AiV-C in their herds.[13]

Prevalence of PKoV/AiV-C decreases as pigs get older.[13,21] In Eastern Africa, prevalence increased as herd size increased and greater virus shedding was found in housed pigs than free range pigs, likely due to increased exposure of animals to virus in manure.[5] Serological studies in the Netherlands detected PKoV/AiV-C in all pigs tested regardless of age.[4]

PKoV/AiV-C is suspected to have caused or contributed to a large outbreak of diarrhea, dehydration, and vomiting in suckling piglets in 10 provinces in China, with morbidity of 80–100% and mortality of 50–90%, beginning in 2010.[8]

4. Transmission

Fecal-oral is the presumed route of transmission, due to high virus concentrations in fecal matter of infected individuals.[22] However, in one study, all piglets in a single herd were found to be PKoV/AiV-C-positive while only 11.8% of sows were shedding virus in feces.[4] Other potential routes of transmission, such as blood, milk, urine, saliva, and aerosols, require further evaluation.[4] Close contact may facilitate cross-species transmission.[1] Wild boars may serve as an important reservoir host and source of infection for domestic swine.[1]

5. Infection in Swine/Pathogenesis

Pathogenesis of PKoV/AiV-C infection is largely unknown due to its relatively recent discovery and the inability to propagate the virus in cell culture.[9,13] PKoV/AiV-C was previously thought to be a gastrointestinal pathogen due to consistent isolation from intestinal samples.[1] Recent reports of viremia have shown PKoV/AiV-C is not restricted to the intestinal tract.[4]

Several hypotheses have been postulated to explain the pathogenesis of PKoV/AiV-C. One suggestion is that virulence of PKoV/AiV-C varies due to the existence of pathogenic and nonpathogenic forms. Another suggests that viral load is important in determining whether a pig will become sick or remain healthy. Yet another suggests that PKoV/AiV-C can only induce diarrhea in a synergistic manner: in the presence of other pathogens like transmissible gastroenteritis virus or rotavirus. Finally, it is possible that PKoV/AiV-C may be an endogenous virus of no clinical significance.[18]

5.1 Clinical Signs

There is limited direct evidence of PKoV/AiV-C causing clinical disease.[5] PKoV/AiV-C infection in swine is primarily a mild diarrheal disease.[1] Piglets less than four weeks are most likely to be infected with PKoV/AiV-C, probably due to an inefficient immune response.[13] Interestingly, one study found that healthy piglets had higher PKoV/AiV-C viral loads than diarrheic piglets, as measured by random-primer sequencing reads of total RNA extracted from directly-collected fecal material.[20] PKoV/AiV-C has been implicated as the cause of an outbreak of diarrhea, dehydration, and vomiting in Chinese piglets, but an etiologic agent has not been definitively determined.[8]

5.2 Postmortem Lesions

PKoV/AiV-C is not considered to cause fatal disease, and postmortem evaluation of tissues is limited. Further research is needed to better understand the pathogenesis of PKoV/AIV-C.

6. Diagnosis

6.1 Clinical History

PKoV/AiV-C should be among the differential diagnoses in any case of diarrhea in suckling piglets less than 28 days old.[23] PKoV/AiV-C may cause dehydration and vomiting in piglets of the same age.[8] Adult pigs are less likely to show PKoV/AiV-C-induced clinical disease.[24]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Reverse transcriptase PCR (RT-PCR), followed by sequence analysis, is widely used for detection and genotyping of KoVs.[1] Consensus primers have been developed for human, bovine, and porcine KoVs at the 3C and 3D region of the genome.[1,25] Electron microscopy has successfully detected KoV.[1] Virus isolation of AiV-1 has been accomplished in BS-C-1 cells and virus can be propagated in Vero cells. The process, however, takes 4–6 weeks.[2,10] AiV-B and PKoV/AiV-C have never been propagated *in vitro*.[1]

An enzyme linked immunosorbent assay (ELISA) has been developed to detect AiV-1 antigen, using monoclonal anti-AiV-1 antibody for capture and anti-AiV-1 antiserum raised in guinea pigs for detection.[9] This diagnostic test has a high sensitivity and specificity.[9]

6.3 Tests to Detect Antibody

Serum neutralization has been used to detect seroconversion of humans to AiV-1.[9] No studies have been conducted evaluating seroconversion to PKoV/AiV-C in pigs.

6.4 Samples

6.4.1 Preferred Samples

Successful detection of PKoV/AiV-C has only been performed using fecal and serum samples from pigs.[4]

6.4.2 Oral Fluids
There is currently no data on the use of oral fluids for PKoV/AiV-C isolation or identification.

7. Immunity

7.1 Post-exposure
Individual piglets can be re-infected over time.[4] Molecular analysis showed that one pig was reinfected with the same PKoV/AiV-C strain at days 3 and 180, and infected with a different PKoV strain at day 36.[4]

Analysis of human serum in Japan found an increased likelihood of seroconversion against AiV-1 with age. Nearly 80% of adults had neutralizing antibody by the age of 35.[9] Prevalence was low in children but increased dramatically between the ages of 25–29 years old to 68% prevalence, likely due to factors influencing frequency of exposure.[9] Antibody titers of 1:8 to 1:32 provided no protection against diarrhea.[9]

7.2 Vaccines
No vaccines have been developed against any KoV to date. Difficulty in propagating KoV in cell culture may be a roadblock to vaccine development, especially for AiV-B and PKoV/AiV-C cluster viruses.[1]

7.3 Cross-protection
Cross protection has not been explicitly examined between KoV strains. Diversity of the VP1 region, which encodes for immunodominant structural proteins, varies enough for subtyping.[10] A study examining the antigenicity of astrovirus and AiV-1 in humans found there was sufficient antigenic distinction and no cross reactivity.[9]

8. Prevention and Control
No anti-viral treatments have been developed for PKoV/AiV-C infection in pigs. Proper sanitation and quarantine of ill pigs should help to prevent and control possible outbreaks.

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code
PKoV/AiV-C is not covered in the 2015 OIE Terrestrial Animal Health Code and there are no recommendations related to importation of swine or pork.

10. Gaps in Preparedness
PKoV/AiV-C is implicated as the cause of diarrheic disease in piglets less than 28 days old. However, little is known about the pathogenesis of PKoV/AiV-C-induced disease. No vaccines are available or currently in development. The hardiness of KoVs in the environment and their association with foodborne disease warrants further investigation. Research into these aspects of the disease could provide better information and tools for the prevention and control of outbreaks.

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Authors, Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University:

- Shane Proietto, BS; 2nd year student,
- Kristin Killoran, PhD; 2nd year student
- Kerry Leedom Larson, DVM, MPH, PhD; Veterinary Specialist

Reviewers, Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University:

- Pamela Zaabel, DVM; Veterinary Specialist
- James A. Roth, DVM, PhD; Director

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