

PORCINE KOBUVIRUS



The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.

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SUMMARY

IMPORTANCE

- Porcine kobuvirus (PKV) is an enteric picornavirus of pigs. It has been found nearly worldwide and occurs both in healthy pigs and pigs with diarrhea. To date, the importance of PKV as a swine pathogen remains unclear.
- Human kobuvirus (Aichi virus) is an emerging cause of gastroenteritis. It seems to be widely distributed in many populations.

PUBLIC HEALTH

- Aichi virus causes gastroenteritis in humans.
- Kobuviruses of humans are genetically distinct from PKV, and PKV has never been isolated from a human diarrhea case.

INFECTION IN SWINE

- PKV has been associated with diarrhea in pigs, but it is also found in healthy animals.
- Most clinical cases involve mild diarrhea. However, outbreaks with high morbidity and mortality have occurred, particularly in piglets. Disease is less common in older animals.
- One experimental study has confirmed PKV as a cause of diarrhea and pathogenic lesions in piglets.
- Co-infection with PKV and other enteric pathogens of swine is very common.

TREATMENT

- There is no treatment for pigs infected with PKV.

CLEANING AND DISINFECTION

- Aichi virus is readily inactivated at 56°C after 20 minutes.
- Kobuviruses are potentially susceptible to disinfection with acids (acetic acid), aldehydes (glutaraldehyde), alkalis (sodium hydroxide), and oxidizing agents like Virkon-S.[®]

PREVENTION AND CONTROL

- Farm sanitation and isolation of sick pigs may help decrease the transmission of PKV.
- There is no PKV vaccine.

TRANSMISSION

- Transmission is thought to be fecal-oral, though other routes may be possible.
- Wild boars might be a source of infection for domestic swine.

PATHOGENESIS

- PKV pathogenesis is poorly understood.
- Reports of viremia have shown that PKV is not restricted to the intestinal tract.

DIAGNOSIS

- Kobuviruses including PKV are difficult to propagate *in vitro*.
- Reverse transcriptase polymerase chain reaction (RT-PCR) assays are used to detect PKV in both feces and serum. PKV RNA has also been detected in oral fluids.
- Antigen capture enzyme linked immunosorbent assays (ELISA) and electron microscopy have been used to detect Aichi virus antigen.
- Serum neutralization tests have been described for Aichi virus and PKV.

EPIDEMIOLOGY

- Kobuviruses infect many different species and are found nearly worldwide.
- Prevalence in domestic pigs ranges from 13–99%.

ETIOLOGY

- PKV is a small, non-enveloped RNA virus in the family *Picornaviridae*.
- There are currently six species in the genus *Kobuvirus*, named *Aichiviruses A–F*.
- PKV belongs to *Aichivirus C*, which contained only swine isolates until recently, when a newly described goat PKV from Korea was assigned to the species.

HISTORY IN SWINE

- PKV was first identified in swine feces in Hungary (2007) and China (2009).
- In the United States, PKV has been detected in feces from nursing piglets in Ohio and intestinal contents/feces from piglets in 15 different states.
- PKV is likely widespread in U.S. swine.

IMMUNITY

- Pigs can be re-infected with the same or different PKV over time.
- There are no vaccines for any kobuvirus.

GAPS IN PREPAREDNESS

- The role of PKV as a swine pathogen remains unclear. Further research is needed on PKV pathogenesis.
- No treatments or vaccines are available.
- Kobuviruses are common in the environment. Their hardiness and association with foodborne disease warrants further investigation.

LITERATURE REVIEW: PORCINE KOBUVIRUS

IMPORTANCE

Porcine kobuvirus (PKV) is an enteric picornavirus of pigs. It has been found nearly worldwide and occurs both in healthy pigs and pigs with diarrhea. Since diarrhea can negatively affect swine welfare, productivity, and profitability, the role of PKV as a swine pathogen needs further evaluation. Additionally, human kobuvirus (Aichi virus) is an emerging cause of gastroenteritis, and it seems to be widely distributed in many populations.

PUBLIC HEALTH

Kobuviruses that infect humans belong to the species *Aichivirus A* (Aichi virus).^{1,2} They are genetically distinct from PKV, which belongs to *Aichivirus C*.¹ Aichi virus was first isolated from the feces of people who consumed raw oysters during the winter months in Japan in 1989.^{3,4} Since then, Aichi virus has been associated with relatively few gastroenteritis outbreaks. However, seroprevalence studies show that Aichi virus antibodies are extremely common in human populations (80–99%).² Symptoms associated with Aichi virus in humans include fever, chills, headache, nausea, vomiting, abdominal pain, diarrhea, and dehydration. PKV has never been isolated from a human diarrhea case.⁵⁻⁷

INFECTION IN SWINE

The clinical importance of PKV is not well understood.⁸ In some studies, PKV has been associated with diarrhea in pigs.⁹⁻¹⁶ In others, the virus has been isolated from healthy animals.^{5,17-21}

Most clinical cases involve mild diarrheal disease.²² However, PKV has been associated with severe disease in at least two outbreaks in China (morbidity 60–100%, mortality 50%–90%).^{15,23} Affected piglets exhibited watery diarrhea, vomiting, and dehydration. Clinical disease becomes less common as pigs age. Adult pigs are the least likely to become clinically ill due to PKV.²⁴

PKV is shed at high rates in pigs between 3–8 weeks-of-age.^{18,24,25} A study by Nantel-Fortier and colleagues²⁶ evaluated PKV shedding throughout all stages of production. Their findings included:

- Increased PKV shedding in late-nursing piglets (6–21 days-old) with diarrhea compared to healthy animals. Piglets shedding PKV at the nursing stage did not shed the virus later in life.
- The highest PKV shedding rate occurred at the post-weaning (nursery) stage.
- Throughout their lifetime, nearly all study pigs (97%) shed PKV at least once.

Experimental infection has been achieved in 10-day-old piglets inoculated with PKV.²⁷ Briefly, feces, serum, and tissue samples were collected from piglets with diarrhea. Tissue samples were positive for PKV, but negative for other common viral and bacterial enteric pathogens. Homogenized tissue samples were combined with Vero cells, and two cell-adapted PKV strains were recovered. One strain (GenBank Number: JQ724539.1) was used to inoculate test pigs. I.M. inoculated-piglets developed diarrhea, emaciation, and nausea at 4 days-post-infection (dpi), and two pigs died at 6 dpi from severe oligohydria. Surviving piglets recovered by 8 dpi.²⁷

Grossly, petechial hemorrhages were seen on the surface of the kidneys.²⁷ Histopathological lesions included:

- *Lung*: bronchial epithelial cells in the pulmonary artery, lymph node-like cells, thickened and congested pulmonary interstitial tissue.²⁷
- *Kidney*: cellular cast formation with many red blood cells, renal tubular epithelial cells exuded in the kidney tubules.²⁷
- *Gastrointestinal system*: marked extravasated blood from the stomach, with lymphocyte and mononuclear phagocyte infiltration of the submucosa; inflammatory cells in the lamina propria of the duodenum; congested blood vessels in the lamina propria and proliferation of goblet cells in the villi intestinalis; lymphocyte infiltration of the rectal submucosa.²⁷

Co-infection with multiple pathogens is common in pigs with diarrhea. Pigs can be simultaneously infected with more than one PKV strain.^{6, 16} PKV has also been found in combination with enteric viruses including porcine astrovirus (PAstV), porcine circovirus (PCV), rotavirus A, transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus (PDCoV) and porcine bocavirus (PBoV).^{10, 15, 16, 20, 28-35} A recent study by Shi and colleagues¹⁶ found that feces from pigs with diarrhea can contain PKV in combination with up to five other swine pathogens (i.e., sextuple infections were documented).

TREATMENT

There is no treatment for pigs infected with PKV.

CLEANING AND DISINFECTION

SURVIVAL

Aichi virus is present in groundwater, sewage, river water, and shellfish.³⁶ Further studies are needed to evaluate the hardiness of PKV in the environment.

DISINFECTION

Aichi virus 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.³⁶ The virus is also resistant to chloroform treatment.⁴

Aichi virus is readily inactivated at 56°C after 20 minutes.³⁶ Temperatures achieved by composting of dairy manure (between 55 and 70°C for at least for three days for a static aerated-pile system) are adequate for inactivation. Kobuviruses, including PKV, are potentially susceptible to disinfection with acids (acetic acid), aldehydes (glutaraldehyde), alkalis (sodium hydroxide), and oxidizing agents like Virkon-S.³⁷

PREVENTION AND CONTROL

DISEASE REPORTING

PKV is not an OIE-listed disease. There are no restrictions for importation of animals from countries or zones affected by PKV. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION

PKV is likely present in many swine farm environments. Seropositivity can be high, and it may not be possible to eliminate PKV. To prevent infection, cleaning and disinfection protocols should be in place. Additionally, sick pigs should be isolated to minimize disease spread.

DISEASE CONTROL

There are no specific control measures for PKV. To minimize risk, standard biosecurity practices should be in place on swine premises.

TRANSMISSION

The major transmission mode is fecal-oral.³⁸ PKV may also spread via contact with feces. PKV viremia has been documented in pigs.³⁹ High PKV prevalence has been seen in piglets where only 12% of sows were shedding virus.⁴⁰ Close contact may facilitate cross-species transmission, and wild boars may serve as a source of infection for domestic swine.²² Cross-species transmission is suspected between cattle, pigs, and sheep.^{5, 7, 41}

PATHOGENESIS

Pathogenesis of PKV is not well understood due to its relatively recent discovery and difficulty in propagating the virus in cell culture.^{6, 42} PKV is found in both healthy and diarrheic pigs. Why some animals become ill, and others do not remains unclear.⁴³ Reports of viremia have shown that PKV is not restricted to the intestinal tract.⁴⁰

DIAGNOSIS

PKV may be suspected in cases of diarrhea, vomiting, and/or dehydration in suckling piglets.^{17, 23}

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

Aichi virus has been isolated in BS-C-1 cells and propagated in Vero cells. The process, however, takes 4–6 weeks.^{4, 36} PKV has rarely been propagated *in vitro*. One report described isolation of the virus from Vero cells combined with homogenized tissue samples from PKV-infected piglets.²⁷ Another detailed isolation of PKV from PK-15 cells inoculated with samples from healthy and diarrheic pigs.⁴⁴ Electron microscopy has also been successfully used to detect kobuvirus.²²

Reverse transcriptase PCR (RT-PCR), followed by sequence analysis, is widely used for identification and genotyping of kobuviruses.²² Consensus primers have been developed for human, bovine, and porcine kobuviruses at the 3C and 3D region of the genome.²²

Examples of assays described for PKV include:

- RT-PCR to detect the conserved 3D gene of PKV^{10, 45, 46}
- RT-loop mediated isothermal amplification (RT-LAMP) to detect the conserved 3D gene of PKV⁴⁷
- TaqMan qRT-PCR to detect the conserved 3D gene of PKV⁴⁸
- Multiplex RT-PCR to detect viral causes of diarrhea in pigs including PEDV, TGEV, and PDCoV (N gene); pseudorabies (PRV-A, VP7 gene), and porcine sapovirus (PSV) and PKV (polyprotein genes).³³
- Multiplex RT-PCR to detect enteric viruses of swine including porcine teschovirus (PTV), porcine torovirus (PToV), PSV, PDCoV, and PAsV.⁴⁹
- Luminex xTAG multiplex detection to detect the M gene of PToV, PEDV, and PDCoV; the RDRP gene of PAsV and PSV; the 3D gene of PKV and PTV; the 5' URT gene of bovine viral diarrhea virus (BVDV); the N gene of TGEV; and the VP6 gene of porcine rubulavirus (PoRV).¹⁶

Next generation sequencing³² and nanopore sequencing⁵⁰ have been described for detection of porcine enteric viruses including PKV. Pretreatment of porcine enteric samples can affect the detection of single-stranded RNA viruses, including PKV, via high-throughput sequencing.⁵¹

An enzyme linked immunosorbent assay (ELISA) has been developed for Aichi virus antigen.⁹ This diagnostic test has high sensitivity and specificity.⁴² No antigen-ELISAs have been described for PKV.

TESTS TO DETECT ANTIBODY

Serum neutralization tests have been developed to detect Aichi virus in humans⁴² and PKV in swine.⁴⁴

SAMPLES

PKV has been detected in fecal and serum samples from pigs.⁴⁰ The presence of PKV RNA has also been confirmed in oral fluids via qRT-PCR.⁵²

EPIDEMIOLOGY

SPECIES AFFECTED

Kobuviruses have been found in humans, pigs,¹⁷ dogs,^{40, 53-57} cats,⁵⁸⁻⁶⁰ rats,^{61, 62} mice,^{63, 64} birds (the European roller *Coracias garrulus*),⁶⁵ ferrets,⁶⁶ rabbits,⁶⁷ goats,⁶⁸⁻⁷⁰ cattle,⁷¹⁻⁷⁵ and sheep.^{76, 77} Bat kobu-like viruses have also been described.⁷⁸⁻⁸⁰

Viruses are assigned to six kobuvirus species, known as *Aichiviruses A–F* (see *Characteristics of Kobuviruses*).¹ In addition to domestic pigs, PKV has been detected in wild boar in Hungary⁸¹ and Serbia,⁸² feral swine in the United States,⁸³ and pygmy hogs in India.⁸⁴ Kobuvirus-contaminated water has also been found.⁸⁵⁻⁸⁷

GEOGRAPHIC DISTRIBUTION

PKV seems to be endemic in many swine herds. The virus has been isolated from pigs in:

- *Asia*: China,^{21, 29, 88} Vietnam,^{11, 61} Thailand,^{9, 89} Japan,⁵ and South Korea⁹⁰
- *Europe*: Hungary,^{17, 91, 92} Italy,²⁴ Czech Republic,⁹⁰ the Netherlands,⁴⁰ Belgium,⁵⁰ Ireland,⁹³ and Serbia⁸²
- *North America*: the United States,^{35, 43, 52} Canada,²⁶ and Mexico³⁰
- *South America*: Brazil^{25, 40}
- *Africa*: Kenya⁹⁴ and Uganda⁹⁴

MORBIDITY AND MORTALITY

PKV prevalence in domestic pigs ranges from 13–99%. Virus has been isolated from both healthy and diarrheic pigs.^{5, 6, 9, 94, 95} Although most cases are mild, outbreaks with high morbidity and mortality have been described (see *Infection in Swine*). In Eastern Africa, PKV prevalence increased with herd size. Higher virus shedding was found in housed pigs compared to free-range pigs, likely due to increased exposure to manure.⁹⁴

ETIOLOGY

CHARACTERISTICS OF PICORNAVIRUSES

Kobuviruses are members of the family *Picornaviridae*. Picornaviruses are small (30 nm), round, single-stranded positive-sense RNA viruses. They have one large open reading frame (ORF) encoding for a single polyprotein that is divided into the nonstructural L protein, three structural capsid proteins (VP0, VP3, and VP1), and seven nonstructural proteins (2A–2C, 3A–3D).^{8, 22, 96} VP1 is highly variable and important for genomic sequence analysis, strain differentiation, and strain identification.²³ The 3D gene, which encodes the RNA-dependent RNA polymerase, is the most conserved region of the genome.^{9, 24}

As of 2020, the family *Picornaviridae* contains 68 genera and 158 species.¹ Additionally, picornavirus "supergroups" have been proposed based on phylogenetic clustering. Kobuviruses belong to SG2, along with the genera *Gallivirus*, *Oscivirus*, *Passerivirus*, *Sakobuvirus*, *Salivirus*, and *Sicinivirus*.⁹⁷ Picornaviruses that infect pigs are found in the genera *Kobuvirus*, *Aphthovirus*, *Cardiovirus*, *Cosavirus*, *Enterovirus*, *Pasivirus*, *Parechovirus*, *Sapelovirus*, *Senecavirus*, and *Teschovirus*.⁸

CHARACTERISTICS OF KOBUVIRUSES

The name kobuvirus comes from the Japanese word *kobu*, meaning "bump," due to the morphological appearance of PKV particles.²² Aichi refers to a prefecture of Japan.

To be classified within a species, isolates must share 70% homology of the polyprotein and P1 aa sequence and 80% homology of the 2C/3CD aa sequence.^{1, 22} Until recently there were three kobuvirus species.

- The type species, *Aichivirus A*, was discovered in 1989 in Japanese patients who developed oyster-associated gastroenteritis.³ Based on genetic sequencing, it was confirmed as a picornavirus in 1998.⁴
- In 2003, bovine kobuvirus was detected in cell culture medium containing calf serum, and later, in sera and feces from healthy cattle in Japan.⁷¹
- PKV was first described in 2008 in the feces of healthy piglets in Hungary¹⁷ (see *History in Swine*). Sequencing showed that PKV was distantly related to both *Aichivirus A* and *B*, and it was identified as a new species within the genus *Kobuvirus*.²²

Currently, there are six species within the genus *Kobuvirus*.¹

- *Aichivirus A* (humans, dogs,^{40, 53} cats,⁵⁸ rats,⁶¹ mice,^{63, 64} and the European roller *Coracias garrulus*)⁶⁵
- *Aichivirus B* (cattle,⁷¹ ferrets,⁶⁶ and sheep)⁷⁶
- *Aichivirus C* (pigs¹⁷ and goats)⁶⁸
- *Aichivirus D* (cattle)⁷²
- *Aichivirus E* (rabbits)⁶⁷
- *Aichivirus F* (bats)⁷⁹

Additionally, three unassigned kobuviruses have been described in bats,^{78, 98} goats,⁶⁸ and Norway rats.⁹⁹

Some PKV strains are missing a 30-aa sequence in the 2B region of the polyprotein gene.¹⁵ This difference has been used to categorize PKVs; group 1 does not contain the deletion and group 2 does. Recently, a third PKV group has been proposed.¹⁵

HISTORY IN SWINE

In 2007, Reuter *et al.*¹⁷ conducted a study to detect porcine caliciviruses in domestic pigs using RT-PCR. Fecal samples were taken from healthy pigs in eastern Hungary. Using agarose gel electrophoresis, an unexpected ~1100-nt nonspecific, single PCR product was found in all samples. Sequencing showed the isolate was a kobuvirus, but genetically distinct from human and bovine strains. Prevalence of PKV in serum and feces from pigs on the same farm was reported to be approximately 60% and 27%, respectively.³⁹ Among pigs less than 3 weeks-of-age, nearly 90% were infected.³⁹ Another PKV was soon after described in China.¹⁰⁰

The first identifications of PKV in the United States occurred in 2012³⁵ and 2013.⁴³

- Sisay *et al.* discovered a PKV in fecal samples from nursing piglets on commercial farms in Ohio. The virus was unintentionally detected using the PSV primer pair SaVXF/R.³⁵ The strain OH/RV50/2011 was sequenced in 2015.¹⁰¹
- Verma and colleagues detected PKV in intestinal contents/feces from both diarrheic and healthy pigs, with the highest prevalence occurring in animals less than 4 weeks-of-age. Isolates were highly similar to PKVs from the Netherlands and Brazil but different from PKVs found in Asia. In all diarrheic pigs, co-infection with either TGEV or rotavirus (A, B, or C) was documented.⁴³
- Unpublished data from the Iowa State University Veterinary Diagnostic Laboratory suggest that PKV is widespread in U.S. swine.⁵²

IMMUNITY

POST-EXPOSURE

Re-infection with PKV has been confirmed in a single pig. The animal was infected with the same PKV strain at days 3 and 180 but infected with a different strain on day 36.⁴⁰

In humans, there is an increased likelihood of Aichi virus seroconversion with age.⁴²

VACCINES

No vaccines have been developed for any kobuvirus to date. Difficulty in propagating kobuviruses in cell culture may be a roadblock to vaccine development, especially for *Aichivirus B* and PKV.²²

CROSS-PROTECTION

It not known whether cross-protection occurs between PKVs. For Aichi virus, diversity of the VP1 region—which encodes for immunodominant structural proteins—varies enough for subtyping.³⁶

GAPS IN PREPAREDNESS

PKV is implicated as a cause of diarrhea in piglets. However, little is known about the pathogenesis of PKV. No treatments or vaccines are available. Additionally, the hardiness of kobuviruses in the environment and their association with foodborne disease warrants further investigation.

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