BUNGOWANNAH VIRUS/ATYPICAL PORCINE PESTIVIRUS

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March 2016

SUMMARY

Etiology

- Pestiviruses are enveloped RNA viruses belonging to the family *Flaviviridae*. The four recognized species include classical swine fever virus (CSFV), bovine viral diarrhea virus types 1 and 2 (BVDV-1, BVDV-2), and border disease virus (BDV) of sheep.
- Bungowannah virus and atypical porcine pestivirus (APPV), two emerging viruses in swine, contain elements of pestiviruses but are highly diverged from other members of the genus.

Cleaning and Disinfection

- The efficacy of disinfectants against Bungowannah virus and APPV is not known.
- Below are EPA-registered products for CSFV, another pestivirus, found on page 26 of: http://www.aphis.usda.gov/animal_health/emergency_management/downloads/fad_epa_disinfectants.pdf.
- Be sure to follow label directions.

EPA Reg. No.	Product Name	Manufacturer	Active Ingredient(s)
211-25	Pheno-Cen Germicidal Detergent	Central Solutions, Inc.	o-Phenylphenol, potassium salt p-tert-Amylphenol, potassium salt Potassium 2-benzyl-4-chlorophenate
211-32	Pheno-Cen Spray Disinfectant/Deodorant	Central Solutions, Inc.	o-Phenylphenol Ethyl alcohol
211-36	Tri-Cen	Central Solutions, Inc.	p-tert-Amylphenol, sodium salt Sodium 2-benzyl-4-chlorophenate Sodium o-phenylphenate
211-50	Q5.5-5.5NPB-2.5HW	Central Solutions, Inc.	Alkyl dimethyl benzyl ammonium chloride Didecyl dimethyl ammonium chloride Octyl decyl dimethyl ammonium chloride Dioctyl dimethyl ammonium chloride
211-62	Low pH Phenolic 256	Central Solutions, Inc.	o-Phenylphenol 2-Benxyl-4-chlorophenol
464-689	Ucarsan Sanitizer 420	Dow Chemical Company	Glutaral
464-696	Ucarsan Sanitizer 4128	Dow Chemical Company	Glutaral
1007-99	Fort Dodge Nolvasan Solution	Zoetis, Inc.	Chlorhexidine diacetate

EPA Reg. No.	Product Name	Manufacturer	Active Ingredient(s)
1007-100	Fort Dodge Nolvasan	Zoetis, Inc.	Chlorhexidine diacetate
1043-26	1-Stroke Environ	Steris Corporation	2-Benzyl-4-chlorophenol o-Phenylphenol 4-tert-Amylphenol
71654-6	Virkon S	E.I. du Pont de Nemours & Company	Sodium chloride Potassium peroxymonosulfate
71847-2	Klor-Kleen	Medentech, Ltd.	Sodium dichloro-s-triazinetrione
71847-6	Klorsept	Medentech, Ltd.	Sodium dichloro-s-triazinetrione

Epidemiology

- In addition to pigs, atypical pestiviruses have been described in cattle, goats, giraffe, pronghorn antelope, bats, and rats.
- Bungowannah virus and APPV are unlikely to affect humans.
- To date, Bungowannah virus has only been detected in Australia and APPV has only been described in the U.S.
- In the New South Wales, Australia, Bungowannah virus outbreak in 2003, pre-weaning mortality up to 35% was observed. Experimentally, Bungowannah virus infection has resulted in post-weaning mortality up to 70% following inoculation at day 35 of gestation. A study of swine tissues from the U.S. found no evidence of Bungowannah virus infection via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).
- A serosurvey of PRRS-positive samples from multiple U.S. states found that 94% contained antibodies to APPV. However, another study found low prevalence (6%) when utilizing qRT-PCR. A recent outbreak of APPV-linked tremors in North Carolina resulted in the death of 700 pigs.

Transmission

- The natural transmission route(s) for Bungowannah virus and APPV are unclear. It has been suggested that the initial introduction of Bungowannah virus in Australia may have been due to vaccine contamination. Aerosols may also play a role in transmission.
- Experimentally, fetuses have been infected with both Bungowannah virus and APPV through direct inoculation in utero. Virus secretion seems to occur mostly in oropharyngeal fluids, but also in nasal secretions, and less frequently in conjunctival secretions and feces.

Infection in Swine/Pathogenesis

- The pathogenesis of Bungowannah virus and APPV is not well understood. Both persistent and chronic infections have been described in pigs experimentally infected with Bungowannah virus.
- Sudden death was the first presentation of Bungowannah virus in the 2003, New South Wales, Australia outbreak. An increase in stillborn and mummification was also observed. Multifocal non-suppurative myocarditis was detected at necropsy. The term porcine myocarditis (PMC) syndrome was used to describe the clinical presentation.
- Piglets affected by congenital tremors experience clonic tremors in the whole body, especially the head and limbs. Posterior paresis and splayleg may also be seen. Recently, APPV-linked tremors have been described in older pigs, 5–14 weeks-of-age.

Diagnosis

- There are no commercially available tests for emerging pestiviruses. Some veterinary diagnostic laboratories may offer testing.
- Diagnostic tests used in a research setting to detect Bungowannah virus/antigen include sequence independent single primer amplification (SISPA), nested RT-PCR, qRT-PCR, virus isolation, in situ hybridization and immunoperoxidase staining. Successful serological tests include the peroxidase linked assay, virus neutralization test, and immunofluorescence assay.
- Diagnostic tests used in a research setting to detect APPV include next-generation sequencing, qRT-PCR, and enzyme linked immunosorbent assay (ELISA).

Immunity

- Little is known about post-exposure immunity. In a study of post-natal infection, pigs seroconverted 10 days after intranasal inoculation with Bungowannah virus.
- There is no vaccine for Bungowannah virus or APPV.
- Bungowannah virus and APPV are highly diverged from other pestiviruses and cross-protection is likely to be limited.

Prevention and Control

- There are no specific prevention and control measures for Bungowannah virus and APPV.

Gaps in Preparedness

- Very little is known about emerging pestiviruses in swine. Further research is needed on transmission, pathogenesis, and the distribution and prevalence of disease. The immune response should be further characterized, in addition to the role that persistent infection may play.
- Diagnostic test development should continue, and vaccine development should be considered. Virus survival in the environment should also be studied, and effective disinfectants should be described.

OVERVIEW

Pestiviruses are enveloped, single-stranded, positive-sense RNA viruses belonging to the family *Flaviviridae*, genus *Pestivirus*. There are four recognized pestivirus species: classical swine fever virus (CSFV), bovine viral diarrhea virus types 1 and 2 (BVDV-1, BVDV-2), and border disease virus (BDV) of sheep. Two novel isolates have been described in swine, Bungowannah virus and atypical porcine pestivirus (APPV). Phylogenetic analysis has shown that both viruses contain elements of pestiviruses but are highly diverged from other members of the genus; as such, Bungowannah virus and APPV have not officially been accepted as pestivirus species.

The only known outbreak of Bungowannah virus occurred on two swine farms in New South Wales, Australia, in 2003. Sudden death occurred in unweaned pigs, and an increase in stillbirths and mummified piglets was also seen. Pre-weaning mortality was observed up to 35%. Multifocal non-suppurative myocarditis was detected at necropsy; the term porcine myocarditis (PMC) syndrome used to describe the clinical presentation. An infectious agent was suspected, but Bungowannah virus was not identified until 2007, when the virus was isolated from pig fetuses that had been experimentally inoculated with infective tissues (collected from fetuses in the 2003 outbreak). Additional studies later confirmed that Bungowannah virus was the causative agent of PMC. Full genome sequencing showed that Bungowannah virus had considerable variation in nucleotide and amino acid sequences compared to other recognized and putative pestiviruses. Testing in the U.S., via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), identified no Bungowannah virus-positives from 64 swine tissue samples collected from the upper Midwest.

APPV was first detected in 2015 via metagenomic sequencing of a porcine reproductive and respiratory syndrome (PRRS)-positive serum sample. Phylogenetic analysis showed the greatest similarity was to a newly described bat pestivirus, described in China in 2012. Later, an enzyme linked immunosorbent assay (ELISA) was developed, targeting APPV Erns, an envelope glycoprotein. Using that test, 94% of PRRS-positive serum samples were found to also contain cross-reactive antibodies to APPV. In 2016, a second APPV was identified in tissues from piglets with congenital tremors (type AII), a syndrome characterized by generalized clonic tremors of the whole body, especially the head and limbs. This virus was found be related to the Chinese bat pestivirus, as well as the first described APPV. A qRT-PCR assay, developed for this study, found a low prevalence of APPV (6%) in grower samples that had been submitted to the Iowa State University Veterinary Diagnostic Laboratory for routine screening purposes. Congenital tremors were reproduced in piglets farrowed by sows inoculated with APPV during gestation. Recently, an APPV was isolated from a pig in North Carolina with uncontrollable shaking; more than 700 affected pigs in the herd died. Notably, this outbreak occurred in pigs 5–14 weeks-of-age, significantly older than piglets in which congenital tremors occur.

In addition to pigs, atypical pestiviruses have been described in cattle, goats, giraffe, pronghorn antelope, bats, and Norway rats. Cross-species transmission of Bungowannah has been achieved experimentally. Maternal and fetal infection occurred in cattle following inoculation during early gestation, and fetuses mounted an antibody response; however, non-pregnant cattle and sheep showed no signs of disease. There is no evidence that pestiviruses are zoonotic, making it unlikely that humans would be affected by Bungowannah virus or APPV. Although pestiviruses have a worldwide distribution, to date, Bungowannah virus has only been detected in Australia, and APPVs have only been found in the U.S. The efficacy of disinfectants against Bungowannah virus and APPV is not known. However, there are EPA-registered products for CSFV that may also be effective against emerging pestiviruses.

The natural transmission route(s) for Bungowannah virus and APPV are unclear. In Australia, Bungowannah virus spread from one farm complex to a second farm complex (within the same production system). On the second farm complex, aerosols generated during decontamination of one

production module may have resulted in re-infection of a virus-free module located 0.5–1.5 km away. It has been suggested that the initial introduction of Bungowannah virus in Australia may have occurred as a result of vaccine contamination. Experimentally, fetuses have been infected with both Bungowannah virus and APPV through direct inoculation in utero. In post-natal pigs inoculated with Bungowannah virus experimentally, virus secretion occurred mostly in oropharyngeal fluids, but also in nasal secretions, and less frequently in conjunctival secretions and feces. It is apparently not known how the pathogenesis of Bungowannah virus and APPV differs from other pestiviruses. Persistent infection has been described in piglets inoculated with Bungowannah virus at day 35 of gestation. Chronic infections have been seen in piglets infected at day 90.

There are apparently no commercially available tests for Bungowannah virus or APPV. A few veterinary diagnostic laboratories (Iowa State, Kansas State) indicate that molecular diagnosis of porcine pestivirus is available on their website. Most information regarding the identification of these viruses comes from the research setting, where a number of diagnostic techniques have been utilized. For Bungowannah virus, independent single primer amplification (SISPA) was used to identify novel viral nucleic acid in fetal serum, and a nested RT-PCR was used to determine the authenticity of the viral nucleic acid sequences developed. In situ hybridization was used to detect virus in fixed tissue, and immunoperoxidase staining has been successfully used to detect viral antigen. Viral isolation has been accomplished in porcine cells (PK-15A), but also in others including those of bovine origin (KOP-R). Several qRT-PCR assays have been described. Serological testing for Bungowannah virus has been completed with the peroxidase linked assay, virus neutralization test, and immunofluorescence assay. Bungowannah virus-specific monoclonal antibodies have also been published, as Bungowannah virus does not cross-react with pan-reactive pestivirus mAbs. Next-generation sequencing was used to first identity APPV. Viral RNA has been detected via qRT-PCR. An ELISA has also been described.

Little is known about immunity to novel pestiviruses. In the New South Wales, Australia, outbreak in 2003, Bungowannah virus continued to circulate until widespread immunity developed in breeding animals. In a study of post-natal infection, clinical signs were largely absent in pigs following intranasal inoculation with Bungowannah virus; however, seroconversion occurred at 10 days post-infection and viremia and viral shedding decreased afterwards. There are no vaccines for Bungowannah virus or APPV. Cross-protection between pestiviruses is unlikely, since Bungowannah virus and APPV show significant genetic variation compared to recognized species such as CSFV.

There are no specific prevention and control measures for Bungowannah virus and APPV. Standard biosecurity practices should be in place to prevent transmission of infectious diseases on swine production sites. Although emerging pestiviruses have been identified as the cause of PMC in Australia and congenital tremors in the U.S., little is known about the pathogenesis of disease. Further research is needed on possible transmission modes of these viruses, as well as the distribution and prevalence of disease. The immune response to emerging pestiviruses is poorly understood, in addition to the role that persistent infection may play. Porcine pestivirus diagnosis may be available at some veterinary diagnostic laboratories; however, there are no commercially available test kits. There is also no vaccine. Virus survival in the environment should be characterized, and effective disinfectants should be described, as they would be critical to an outbreak response.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Pestiviruses are enveloped, single-stranded, positive-sense RNA viruses belonging to the family *Flaviviridae*, genus *Pestivirus*. Four pestivirus species are currently recognized according to host range, including classical swine fever virus (CSFV), bovine viral diarrhea virus types 1 and 2 (BVDV-1, BVDV-2), and border disease virus (BDV) of sheep. Recently described swine isolates, Bungowannah virus and atypical porcine pestivirus, have not been approved as pestivirus species.[1]

Additional putative pestiviruses have been described[2] including:
- Atypical pestivirus, isolated from a giraffe with mucosal-like disease, Kenya, 1969;[3]
- HoBi/atypical pestivirus/BVDV-3, isolated from fetal calf serum, Brazil, 2004[4] (additional atypical bovine pestiviruses have since been described);[5,6]
- BDV-2, isolated from caprine fetuses (from a mixed goat/sheep flock), Italy, 2005;[7]
- Atypical pestivirus, isolated from a blind, immature pronghorn antelope, Wyoming, U.S., 2005;[8]
- Atypical pestivirus, isolated from bats, China, 2012;[9] and
- Atypical pestivirus, isolated from Norway rats, New York City, U.S., 2014.[10]

Classification schemes are based on genetic sequencing and phylogenetic analysis. A heterologous complementation method has also been described, in which genomic regions encoding pestivirus structural proteins and their exchangeability were examined, and phylogenetic relationships were clarified.[11]

1.2 Strain Variability

1.2.1 Bungowannah Virus

Bungowannah virus was first described in association with an increase in stillborns and high mortality in weaners (3–4 weeks old) on two pig farms in New South Wales, Australia, in 2003.[12] Postmortem lesions showed that the heart was primarily affected, and the term porcine myocarditis syndrome (PMC) was coined. At that time, the causative virus was unidentified; however, in 2007, Kirkland et al. identified a novel pestivirus in pooled sera from 12 experimentally infected pig fetuses.[13] The isolate, named Bungowannah virus, was found to contain significant differences in the 5ꞌUTR, Npro and E2 coding regions compared to other known pestiviruses. Phylogenetic analysis showed that Bungowannah virus was only distantly related to most other pestiviruses, including CSFV, and had greatest similarity to a recently described pronghorn antelope isolate.[13]

Studies were later conducted to confirm that Bungowannah virus caused PMC. In 2007, Finlaison et al. collected serum samples from gilts and sows from farms affected and unaffected by PMC, as well as serum, tissues (including the heart) and pericardial/pleural fluid from stillborn pigs. A temporal relationship was observed between PMC occurrence on a farm and serological response to Bungowannah virus in gilts, sows, and stillborn pigs.[14] It was also demonstrated that large amounts of Bungowannah virus RNA were detectable in tissues from stillborn pigs, including the myocardium.[14] Finlaison et al. sought to further investigate Bungowannah virus infection in 2010, with a study involving direct inoculation of tissues from cases of PMC into porcine fetuses of varying ages in utero.[15] Experimental infection in pregnant sows was also characterized in 2010; as cited in Kirkland et al., clinical outcomes ranging from stillbirth and mummification to persistent and chronic infection were observed.[16] A follow-up study, conducted in 2012, focused on post-natal infections in swine. After intranasal inoculation with Bungowannah virus, clinical signs were largely absent; however, viremia and viral shedding in oropharyngeal secretions were detected at 3 days post-infection, and seroconversion occurred at 10 days post-infection.[17]

The whole genome of Bungowannah virus was sequenced in 2015 by Kirkland et al. While the virus contained the structural and non-structural proteins of a pestivirus, it also showed considerable variability in nucleotide and amino acid sequences when compared to other recognized and putative pestiviruses.[18] In this study, a set of novel mAbs were also generated to allow Bungowannah virus detection, since there was no reactivity when the virus was combined with pan-reactive pestivirus mAbs.[18]

1.2.2 Atypical Porcine Pestivirus
In 2015, a novel pestivirus was identified in five serum samples from pigs involved in a porcine reproductive and respiratory syndrome virus (PRRSV) metagenomics sequencing study conducted by Hause et al.[19] As with Bungowannah virus, the detected APPV isolates were found to be significantly diverged from most other known pestiviruses. Phylogenetic analysis showed the greatest similarity was to a newly described pestivirus in bats (*Rhinolophus affinis)* from China, detected in 2012,[9] with 68% pairwise similarity. Only 25–28% pairwise similarity to the more well-known pestiviruses (CSFV, BVDV-1 BVDV-2, and BDV) was found.[19] An enzyme linked immunosorbent assay (ELISA), using recombinant APPV Erns (an envelope glycoprotein), further revealed that 94% of PRRSV-positive serum samples from multiple states contained cross-reactive antibodies to APPV.[19]

A study by Arruda et al. published in 2016 also identified an APPV, this time from piglets with congenital tremors (type AII).[20] This virus was closely related to the Chinese bat pestivirus and APPV described above. Samples from growers submitted to the Iowa State University Veterinary Diagnostic Laboratory for routine testing were screened for APPV RNA via RT-PCR; only 6% (22/362) animals tested positive.[20] In this study, pregnant sows were also inoculated with APPV (intravenous, intranasal, and inoculation of fetal amniotic vesicles), in an attempt to induce disease. Inoculated sows farrowed pigs with congenital tremors while controls did not; APPV was also consistently detected in tissues from affected piglets via RT-PCR.[20]

Recently, an APPV was isolated from a pig in North Carolina with uncontrollable shaking; more than 700 affected pigs in the herd died. Notably, this outbreak occurred in pigs 5–14 weeks-of-age, significantly older than piglets in which congenital tremors occur.[21]

2. Cleaning and Disinfection

2.1 Survival
No published information is available regarding the survival of Bungowannah virus or APPV. Survival of CSFV, another pestivirus affecting swine, has been described by the World Organization for Animals Health (OIE) as follows:
- *In the environment:* moderately fragile and does not persist in the environment; sensitive to drying and ultraviolet light; survives well in pens during cold conditions (up to 4 weeks in winter); survives 3 days at 50°C and 7–15 days at 37°C.[22]

- *In meat/tissues:* survives in meat during salt curing and smoking for 17 to >180 days depending on the process used; persists 3–4 days in decomposing organs; persists 15 days in decomposing blood and bone marrow.[22]

2.2 Disinfection
No published information is available regarding the disinfection of Bungowannah virus or APPV. Generally, aldehydes (e.g., formaldehyde, glutaraldehyde) are highly effective against enveloped viruses. Additional effective disinfectants may include acids, alcohols, alkalis, halogens (e.g., hypochlorite, iodine), and oxidizing agents (e.g., peroxyacetic acid, Virkon-S®). Biguanides (e.g., chlorhexidine),

phenolic compounds, and quaternary ammonium compounds (e.g., Roccal) have limited efficacy against enveloped viruses.[23]

Disinfection of CSFV, another pestivirus affecting swine, has been described by the World Organization for Animals Health (OIE) as follows:

- *Reportedly susceptible to:* ether, chloroform, ß-propiolactone (0.4%)

- *Reportedly inactivated by:* chlorine-based disinfectants, cresol (5%), sodium hydroxide (2%), formalin (1%), sodium carbonate (4% anhydrous or 10% crystalline, with 0.1% detergent), ionic and non-ionic detergents, and strong iodophors (1%) in phosphoric acid.[22]

There are EPA-registered products for CSFV that may also be effective against emerging pestiviruses. Product names can be found on page 26 of the document *Potential Pesticides to Use Against the Causative Agents of Selected Foreign Animal Diseases in Farm Settings,* available at: http://www.aphis.usda.gov/animal_health/emergency_management/downloads/fad_epa_disinfectants.pdf.

Be sure to follow label directions.

3. Epidemiology

3.1 Species Affected
Pestiviruses are known to affect many species of wild and domestic ruminants, as well as pigs. Atypical pestiviruses have been described in pigs, cattle, goats, giraffe, pronghorn antelope, bats, and Norway rats.

As cited in Kirkland et al., sheep and cattle have been experimentally infected with Bungowannah virus. Non-pregnant animals showed no signs of disease. Maternal and fetal infection occurred following inoculation of pregnant cattle in early gestation; all infected fetuses mounted an antibody response.[16]

3.2 Zoonotic Potential
There is no evidence that pestiviruses are zoonotic. Humans are unlikely to be affected by Bungowannah virus or APPV.

3.3 Geographic Distribution
In general, pestiviruses have a worldwide distribution. As previously noted, Bungowannah virus has been detected in Australia[13,14] and APPV has been detected in the U.S.[19,20] A serological study revealed that APPVs may be widespread in the U.S. swine population.[19] Congenital tremors (type AII), a disease recently linked to APPV, is also sporadically found in many swine producing countries and likely throughout the world.[20]

3.4 Morbidity and Mortality
In the New South Wales, Australia, outbreak of Bungowannah virus that occurred in 2003, high seroprevalence was seen in breeding sows in production units where disease had occurred. More than 50,000 pigs died.[16] Pre-weaning mortality was observed up to 35%.[16] As cited in Kirkland et al., an experimental study of Bungowannah virus infection at days 35 and 90 of gestation resulted in post-weaning mortality of 70 and 29% respectively.[16] A study of U.S. swine found no positives in 64 tissue samples, collected from the upper Midwest from 2007–2010, using qRT-PCR.[24]

Serological testing has revealed that 94% of PRRSV-positive swine serum samples from multiple U.S. states contained cross-reactive antibodies to APPV.[19] However, a study of grower samples (submitted to the Iowa State University Veterinary Diagnostic Laboratory for routine diagnostic purposes) found that

only 6% were positive for APPV by qRT-PCR.[20] An outbreak of tremors, resulting in the death of more than 700 pigs, was recently described.[21]

4. Transmission

The natural transmission route(s) for Bungowannah virus and APPV are unclear. In New South Wales, Australia, Bungowannah virus outbreak that occurred in 2003, clinical cases were first observed in a single production module on the index farm; however, after 4–6 weeks, disease was detected in other modules on the same farm and on another farm 60km away. After the initial outbreak subsided in late 2004, Bungowannah virus remained endemic on both farm complexes. The virus was later eradicated from the first farm.[16] Eradication efforts on the second farm were not successful, and it was suspected that the virus was re-introduced through aerosols generated during decontamination of an infected module located 0.5–1.5 km from the virus-free modules.[16] The outbreak likely ended when naïve pigs were no longer available.[12,16] As cited in Kirkland et al., it has been suggested that the virus may have been introduced as a vaccine contaminant.[16]

Experimentally, fetuses have been infected with both Bungowannah virus and APPV through direct inoculation in utero.[13,20] In one study, post-natal pigs intranasally inoculated with Bungowannah virus showed few clinical signs, although viremia was detected and virus secretion occurred (mostly in oropharyngeal fluids, but also in nasal secretions, and less frequently in conjunctival secretions and feces).[17] Experimentally, the infectious dose for intranasal inoculation was found to be $1.6–3.2\log_{10}$ $TCID_{50}$.[17] In another study, at the time of amniotic vesicle inoculation with APPV, sows were simultaneously inoculated with APPV intravenously and intravenously; however, none developed clinical signs, and neither viremia nor virus was detected via RT-PCR.[20]

5. Infection in Swine/Pathogenesis

5.1 Pathogenesis

It is apparently not known how the pathogenesis of Bungowannah virus and APPV differs from other pestiviruses. Although Bungowannah virus has been linked to PMC, the pathogenesis of observed myocarditis and myocardial necrosis is unclear.[14] Similarly, the mechanism for central nervous system dysfunction in most APPV-infected pigs with congenital tremors is unknown.[20] In an experimental study, nearly all tissues from APPV-inoculated piglets had similar levels of detectable RNA, making the specific site of replication unclear.[20]

5.2 Pestiviruses and Persistent Infection (PI)

A unique aspect of pestiviruses is their ability to avoid immune detection and establish persistent infections (PI). When infection of a naïve pregnant animal occurs during a particular range of timepoints within the first trimester, pestiviruses including CSFV, BVDV, and BDV are known to establish persistent infections in offspring.[25] Research has shown that two viral proteins unique to pestiviruses are necessary for the establishment of persistent infection. Npro and Erns, a non-structural protein and a viral envelope protein containing an intrinsic RNase, are non-redundantly necessary for inhibition of type 1 IFN response in infected cells.[26] Because all known pestiviruses contain these two proteins, it is possible to extrapolate that novel pestiviruses may likewise have the ability to establish PI in fetuses in infected herds.

5.2.1 Classical Swine Fever Virus and PI

Persistent infection is a well-known consequence of infection with CSFV in both domestic pigs and wild boar. Pigs infected with strains showing low virulence have been known to shed virus for several months, and pregnant sows infected with a low virulence strain can transplacentally infect fetuses with the virus. Early studies showed that infection of a naïve sow at days 45 or 60 of pregnancy resulted in PI offspring. Infection at day 90 resulted in a mixed litter with some offspring being PI, and others having mounted an

immune response and cleared the virus.[27-29] These results are consistent with the window of infection seen for PI caused by BVDV in calves.

5.2.2 Bovine Viral Diarrhea Virus and PI

In cattle, in utero infection with a non-cytopathic strain of BVDV, another member of the genus *Pestivirus*, can lead to PI if the fetus is exposed between days 18–125 of gestation.[30] Animals that are PI are often born healthy,[31] although some show stunted growth and poor response to disease challenge.[32] Furthermore, these animals release large amounts of virus in secretions and excretions, including milk, saliva, urine, and feces.[33]

Only persistently infected animals are at risk for developing mucosal disease—a fatal complication of BVDV infection. Mucosal disease is characterized by erosions in/on the mouth and/or coronary band; profuse diarrhea; and death typically within 3–10 days of the onset of symptoms. Death is often the first and only symptom of mucosal disease. Mucosal disease develops when an animal infected with a non-cytopathic strain of BVDV encounters a cytopathic strain—either from within (mutation of the strain already circulating within the animal) or from outside (such as from a herd-mate acutely infected with a cytopathic strain). Because the animal has no immune response to BVDV, the cytopathic ("cell-destroying") strain is able to reproduce unchecked, leading to the severe clinical signs and death in a short period.[33]

In addition to PI, infections in utero may result in embryonic death or congenital malformations such as cerebellar hypoplasia. Infection with BVDV has also been linked to decreased milk production, reproductive dysfunction, slowed growth, increased occurrence of other disease, unthriftiness, and early mortality in cattle. Although the economic effect is difficult to quantify, it has been estimated that losses at the national level range between $10–40 million per million calvings.

5.2.3 Bungowannah Virus/Atypical Porcine Pestivirus and PI

As cited in Kirkland et al., piglets that were infected with Bungowannah virus at day 35 of gestation were shown to be PI.[16] In addition, chronic infections were seen in piglets infected at day 90; in these pigs, viremia was detected for up to 7 months.[16] The potential role of PI in APPV transmission and virus maintenance in a swine herd is unclear. It has been suggested that the lack of antibody response observed in some APPV-infected fetuses with PMC could be related to PI.[14] The potential role of PI or other immune dysfunction should also be examined in relation to the clinical expression of congenital tremors or other central nervous system dysfunction.[20]

5.3 Clinical Signs

During the 2003 Bungowannah virus outbreak in New South Wales, Australia, sudden death was first observed in unweaned piglets. A large increase in the number of stillbirths, and a more moderate rise in the number of mummified piglets, were observed soon after.[16] At that time, the case definition was the occurrence of four or more affected piglets born dead in a litter.[12] Affected sows largely showed no obvious clinical signs. Of piglets born alive, many (up to 35%) died within three weeks; however, most showed no signs of disease. Some clinically affected piglets developed cyanosis of the snout and ears, with vocalization and hyperventilation observed before death.[12,16] Death was also seen in some post-weaning pigs following handling or transport.[12] No clinical signs were observed in older pigs, including weaners and growers.

Congenital tremors (also known as myoclonia congenita, trembles, or jumpy pig disease) cause generalized tremors in piglets involving the whole body, especially the head and limbs.[34] The intensity of tremors is related to arousal and/or activity level. Clonic tremors occur in the skeletal muscles, and posterior paresis and splayed hindlegs may be seen. Tremors linked to APPV have recently been described in 5–14 week-old-pigs.[21]

5.4 Postmortem Lesions

In the 2003 outbreak of Bungowannah virus in New South Wales, Australia, most stillborn piglets showed few gross lesions. Gross findings at necropsy included moderate subcutaneous edema; excessive pericardial, pleural, and peritoneal fluid; bilateral cardiac dilation and ecchymotic hemorrhages in the myocardium; and venous congestion in the liver.[12,16] Irregular areas of increased pallor were sometimes found in the heart. Fibrin tags were observed in some cases on the thoracic and abdominal viscera. Histologically, the most consistent finding in affected piglets was acute to subacute multifocal nonsuppurative myocarditis with myonecrosis. Some piglets also showed nonsuppurative interstitial pneumonia, encephalitis, hepatitis, and lymphadenitis. Myocardial fibrosis occurred in some older, unweaned pigs.[12,16] In an experimental study, myocarditis was associated with infection during late gestation.[15]

In pigs with congenital tremors, hypomyelination or demyelination of the brain and spinal cord occurs. Reduced spinal cord lipid content is also associated with congenital tremors type AII, as is depressed or aberrant cerobroside synthesis.[34]

6. Diagnosis

There are apparently no commercially available tests for Bungowannah virus or APPV. A few veterinary diagnostic laboratories (Iowa State, Kansas State) indicate that molecular diagnosis of porcine pestivirus is available on their website. Most information regarding the identification of these viruses comes from the research setting, where a number of diagnostic techniques have been utilized. The following information summarizes the major papers that have been published on identification of Bungowannah virus and APPV.

6.1 Bungowannah virus

In the New South Wales, Australia, PMC outbreak that occurred in 2003, researchers used sequence independent single primer amplification (SISPA) to identify novel viral nucleic acid in fetal serum, and a nested RT-PCR was used to determine the authenticity of the viral nucleic acid sequences developed.[13] A qRT-PCR method was later developed and published. In situ hybridization was used to localize Bungowannah virus RNA in fixed tissue;[14] immunoperoxidase staining has also been successfully used to detect viral antigen. Bungowannah virus is amenable to virus isolation, and will grow in porcine cell lines, such as PK-15A, over several passages with little to no decrease in titer.[13] The virus has also been successfully replicated in cell lines of bovine origin (KOP-R line, in particular).[11]

Serological testing for Bungowannah virus has been completed with peroxidase linked assay (PLA),[13] virus neutralization test (VNT),[17] and immunofluorescence assay (IFA).[18] Bungowannah virus-specific monoclonal antibodies have also been published, as Bungowannah virus does not cross-react with pan-reactive pestivirus mAbs.[18] Samples tested have included serum, pleural and pericardial fluid, and formalin-fixed paraffin-embedded heart tissue from still-born piglets.[13,14]

To determine the prevalence of Bungowannah virus in pigs in the Midwestern U.S., three qRT-PCR assays were developed based on the highly conserved 5´UTR region, the Npro gene, and the E2 envelope protein gene. To increase the likelihood of detection, samples were taken from recent cases of respiratory disease or abortion with evidence of myocarditis; no Bungowannah virus was detected in the samples chosen for testing. It should be noted, however, that as no Bungowannah-positive material was available for validation of the tests, the positive control was synthesized from a GenBank accession.[24]

6.2 Atypical Porcine Pestivirus

Following the identification of novel pestiviruses in bats and rats by next-generation sequencing, researchers used metagenomics sequencing to identify a novel virus, APPV, in swine in the U.S. An ELISA was developed for the detection of APPV Erns peptides, a protein only found in pestiviruses.[19] Using a combination of next-generation sequencing and qRT-PCR (targeting NS3), an APPV was isolated from pigs with congenital tremors. Preferred specimens were brainstem, mesenteric lymph node, tracheobronchial lymph node, and whole blood.[20]

7. Immunity

7.1 Post-exposure

In the New South Wales, Australia, outbreak in 2003, Bungowannah virus continued to circulate until widespread immunity developed in breeding animals.[16] In a study of post-natal infection, clinical signs were largely absent in pigs following intranasal inoculation with Bungowannah virus; however, seroconversion occurred at 10 days post-infection and viremia and viral shedding decreased afterwards.[17] As cited in Kirkland et al., piglets with chronic Bungowannah virus infection may be viremic for up to 7 months.[16] No information is available regarding post-exposure immunity and APPV.

7.2 Vaccines

There is no vaccine for Bungowannah virus or APPV.

7.3 Cross-protection

Bungowannah virus and APPV are highly diverged from the recognized pestivirus species CSFV, BVDV-1, BVDV-2, and BDV. The failure of Bungowannah virus to react with pan-reactive pestivirus mAbs has been demonstrated experimentally.[18]

8. Prevention and Control

Standard biosecurity practices should be in place to prevent transmission of infectious diseases on swine production sites. These include isolation of new stock and sick animals; controlling farm access by wildlife, birds, and rodents; maintaining a closed herd with 'all in/all out' production; shower-in, shower-out of employees; limiting human visitors; and cleaning and disinfection.

In the New South Wales, Australia, outbreak in 2003, the two affected farms were quarantined following the identification of Bungowannah virus. Following depopulation and disinfection the virus was eliminated from the first farm, but eradication efforts on the second farm failed. Although the virus was eliminated from several production units at the second farm, virus was thought to be re-introduced through aerosols generated during decontamination of an infected module located 0.5–1.5 km from the virus-free modules.[16]

Pestivirus treatment is not likely to be practical. However, an aromatic cationic compound, known as DB772, has previously been shown to inhibit replication of BVDV-1 in vitro. In a recent study, DB772 was also shown to effectively inhibit BVDV-2, BDV, HoBi virus, pronghorn virus, and Bungowannah virus at concentrations >0.20μM.[35]

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

Bungowannah virus and APPV are not included in the 2015 OIE Terrestrial Animal Health Code. There are no restrictions for importation of animals from countries or zones infected with these viruses.

10. Gaps in Preparedness

Although emerging pestiviruses have been identified as the cause of PMC in Australia and congenital tremors in the U.S., little is known about the pathogenesis of disease. Further research is needed on possible transmission modes of these viruses, as well as the distribution and prevalence of disease. The immune response to emerging pestiviruses is poorly understood, in addition to the role that persistent infection may play. Porcine pestivirus diagnosis may be available at some veterinary diagnostic laboratories; however, there are no commercially available test kits. There is also no vaccine. Virus survival in the environment should be characterized, and effective disinfectants should be described, as they would be critical to an outbreak response.

ACKNOWLEDGEMENTS

To cite:
Leedom Larson K, Pettit K, Zaabel P. Bungowannah virus/atypical porcine pestivirus. Swine Health Information Center and Center for Food Security and Public Health, 2016. http://www.cfsph.iastate.edu/pdf/shic-factsheet-atypical-porcine-pestivirus.

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