ORTHOREOVIRUS

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SUMMARY

Etiology

- Orthoreoviruses are non-enveloped, segmented RNA viruses in the family *Reoviridae*.
- There are 5 known species in the genus *Orthoreovirus*, of which species I is referred to as mammalian orthoreovirus (MRV) or mammalian reovirus. Within this species, there are four distinct serotypes.
- Serotypes 1-3 of MRV, as well as antibodies to each, have been detected in swine.
- A novel pathogenic strain of serotype 3 (MRV3) was reported in U.S. swine herds in 2015.

Cleaning and Disinfection

- Some porcine MRV isolates are susceptible to one hour of 50˚C heat (122˚F); however, the recently discovered U.S. strains are stable at 56˚C (133˚F) and remain viable after exposure to 80˚C (176˚F) or 90˚C (194˚F) for up to one hour.
- Specific information on the disinfection of porcine MRV is lacking. Orthoreoviruses are generally stable over a wide pH range and resistant to ether, chloroform, and lipid solvents. They are potentially susceptible to 0.1% sodium deoxycholate.

Epidemiology

- Orthoreoviruses are ubiquitous, infecting a large variety of animal species over a wide geographic range. In addition to pigs, evidence of orthoreovirus infection has been seen in cattle, sheep, horses, goats, primates, bats, birds, dogs, cats, rabbits, rodents, marsupials, civet cats, and reptiles.
- Zoonotic transmission of orthoreoviruses is possible, although sustained human-to-human transmission has not yet been observed. Illness is most commonly mild and self-limiting; however, more severe respiratory or neurologic forms of the disease have occurred. There have been no reports of porcine MRV infecting humans.
- While MRV is geographically widespread, infection of swine has only been reported in China, Japan, South Korea, and the U.S.

Transmission

- Transmission of MRV in pigs is known to occur by the fecal-oral route. The virus has also been detected in nasal secretions, respiratory aerosols, and fetal tissues.

Infection in Swine / Pathogenesis

- Enteric disease is observed in swine infected with some strains of MRV. The virus replicates in enterocytes, is passed in the feces, and can be spread in contaminated food or water. Along with enteritis, pneumonia and reproductive failure have also been reported in infected pigs. Encephalitis may occur, but appears to be uncommon.

Diagnosis

- Chicken embryos and various cell lines, including baby hamster kidney (BHK-21) cells, African green monkey kidney (Vero) cells, fetal rhesus monkey kidney cells (TF-104), and primary porcine kidney cells, can be used to isolate MRV.
- Reverse transcription polymerase chain reaction (RT-PCR) with primers specific to the S1 and L1 gene is used for diagnosis of MRV3 in swine.
- All mammalian orthoreoviruses share a common group antigen that is utilized for diagnosis by complement fixation, immunofluorescence assay (IFA), and immunodiffusion. Direct and indirect IFA specific to MRV3 are also available. The National Veterinary Services Laboratory (NVSL) in Ames, Iowa currently offers virus isolation and IFA for diagnosis of reovirus in swine.
- Serum neutralization or hemagglutination inhibition (HI) are necessary for serotype identification.

Immunity

- Antibodies capable of inhibiting hemagglutination may appear as early as one week post-infection and generally peak around 11–21 days.
- Young pigs are more susceptible to MRV infection than immunocompetent adults and are thought to be most at risk when maternal antibodies wane at up to 11 weeks of age.
- There is no vaccine for MRV in swine or other animals, including humans.

Prevention and Control

- There is no specific information available for control of MRV in swine.
- Sanitation, quarantine of new animals, and periodic serologic testing are effective at preventing MRV infection in laboratory rodent colonies.
- Common swine industry biosecurity practices should be in place.

Gaps in Preparedness

- Continued research on the epidemiology of MRV and its interaction with other enteric pathogens of swine is needed.
- Existing biosecurity measures may need to be modified to account for novel pathogenic strains with greater thermostability.

OVERVIEW

Mammalian orthoreoviruses (MRVs) are widespread and infect many animal species. Like influenza, their segmented genome allows for reassortment in nature and contributes to their genetic diversity. Of the four known MRV serotypes, only the first three are known to infect swine and humans. Despite their broad host tropism, MRVs are generally thought to produce mild, self-limiting disease. Enteric and respiratory illness are most commonly observed in infected swine, often preferentially affecting younger animals. Recently, a novel pathogenic strain of serotype 3 (MRV3) has surfaced in North Carolina, Minnesota, and Iowa. The thermostability and trypsin-resistance of MRV3 may play a role in outbreaks of acute gastroenteritis in piglets, either alone or in conjunction with other enteric pathogens, such as porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV). Co-infection has not been studied yet, and the U.S. isolates of MRV3 are capable of producing disease experimentally.

While MRV can be found throughout the world, epidemiology of the disease in swine is not well understood. Cases have only been reported in China, South Korea, Japan, and most recently the U.S. Clinical signs may include fever, inappetence, weight loss, diarrhea, enteritis, nasal discharge, cough, and encephalitis. Piglets appear to be affected more frequently and severely, although disease has occurred in adult pigs as well. In the U.S., enteric disease in piglets can result in high morbidity and mortality, resulting in an urgent need to understand the role of pathogenic MRV strains in these outbreaks. Zoonotic transmission of MRV has also occurred, although not specifically from pigs to humans. Continued surveillance and epidemiological studies are needed to understand the full scope of the disease.

Diagnosis can be achieved by virus isolation from fecal or tissue samples using a variety of cell lines. Complement fixation, immunofluorescence, and immunodiffusion assays are used for the detection of viral antigens, in addition to reverse transcription polymerase chain reaction (RT-PCR). Serum neutralization or hemagglutination inhibition (HI) are necessary for serotype identification. Enzyme immunoassay with an MRV3 antigen can also be used to detect antibodies to serotypes 1-3, the three major MRV serotypes.

Detectable levels of MRV in feces and nasal secretions can appear as soon as 24 hours after exposure. Neutralizing antibodies are serotype specific and offer some protection from infection. Maternal antibodies wane as late as 11 weeks, leaving piglets more susceptible after this time. In the absence of a vaccine for pigs, protection depends on maternal antibody and strict biosecurity measures. The virus is stable at high temperatures over a wide pH range, and existing industry practices may be insufficient to contain its spread. In light of the recent discovery of pathogenic MRV isolates in swine herds and ring-dried swine blood meal in the U.S., as well as the economic consequences of enteric disease outbreaks, additional research is essential.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Mammalian orthoreoviruses (MRV), still commonly referred to simply as reoviruses, are non-enveloped viruses with double stranded RNA genomes that are divided into ten distinct segments. They are classified in the genus *Orthoreovirus* of the *Reoviridae* family. Within the genus *Orthoreovirus*, species I of V is known as mammalian reovirus.[1] The mammalian reovirus species can be further broken down into four distinct serotypes, determined by the cell attachment protein, σ1.[2] Viruses of the genus *Orthoreovirus* are also divided into two phenotypic groups – fusogenic and non-fusogenic. Species II – V are fusogenic and capable of forming giant syncytial cells. Species I is non-fusogenic and generally considered to be of less pathological consequence.[3] However, a novel porcine serotype 3 mammalian orthoreovirus (MRV3) was recently discovered in pigs with diarrhea in the U.S., warranting further investigation into the potential role of MRV in swine disease outbreaks.[4]

1.2 Strain Variability

Genetic reassortment occurs within each genus or serogroup of reovirus, due to the segmented nature of the genome,[1] and reassortant viruses have been produced *in vitro* by coinfection of cells or mice with two MRVs from different serotypes.[2] This contributes to the molecular diversity of orthoreoviruses and their ability to increase in virulence and broaden their host range.[4] The S1 genome segment, which encodes the serotype-determining σ1 attachment protein, shows the greatest diversity among molecularly characterized MRV strains. Serotypes 1 through 3 of mammalian reoviruses have been isolated from swine,[5] although pathogenicity of type 2 remains uncertain.[6]

2. Cleaning and Disinfection

2.1 Survival

Thermostability of MRV varies by strain.[2] Initial laboratory studies indicate that the new U.S. strains of MRV3 in swine are stable at 56˚C (132.8˚F) and remain viable after exposure to 80˚C (176˚F) or 90˚C (194˚F) for up to one hour. They are also trypsin-resistant.[4] Some porcine MRV isolates are susceptible to one hour of 50˚C (122˚F) heat.[7] The virus is also generally stable over a wide pH range,[1] including acidic conditions with a pH as low as three.[7] In an environment with high relative humidity, MRV can survive in aerosols.[2]

There is some evidence to suggest that serotype 2 mammalian orthoreovirus (MRV2) infection in humans is more prevalent in the summer months in North America,[2] but similar studies have not been done in swine.

2.2 Disinfection

Orthoreoviruses are non-enveloped and resistant to lipid solvents,[1] ether, and chloroform, but sensitive to 0.1% sodium deoxycholate.[7]

3. Epidemiology

3.1 Species Affected

Evidence of orthoreovirus infection has been seen in a wide range of species, including swine, cattle, sheep, non-human primates, bats, birds[1], dogs, cats, goats, rabbits, rodents, marsupials,[2] civet cats, and reptiles.[4] Often they produce no clinical signs; however, respiratory and/or enteric disease has been reported in swine, cattle, sheep, horses, and dogs. Reoviruses have also been isolated from healthy swine, as well as aborted fetuses, stillborn, mummified, and weak live-born pigs.[7] Both hepatitis and meningitis

have been observed in infected primates. Despite the presence of the virus in many species, disease causality continues to be debated.[1]

3.2 Zoonotic Potential

Non-fusogenic MRVs have been associated with mild, self-limiting respiratory and gastrointestinal illness in humans. Transmission of fusogenic orthoreoviruses from bat reservoirs to humans has also been reported in cases of more severe, acute respiratory illness. Epidemiological tracing indicates that human-to-human transmission likely occurred in some of these cases, though sustained transmission in human populations has not been observed.[3] There are limited reports of MRVs causing neurologic disease in humans, and it's hypothesized that protective immunity generated by non-neurovirulent strains may limit the number of cases that are seen.[8] Zoonotic transmission specific to the newly isolated MRV3 isolates from U.S. swine has not been reported.

3.3 Geographic Distribution

Cases of MRV3 associated with diarrhea in swine have previously been reported in southern China[9] and South Korea.[10] However, until 2013, there were no reports of similar disease in the U.S. Investigation into outbreaks of porcine epidemic diarrhea virus (PEDV) revealed the presence of MRV3 on farms in North Carolina, Minnesota, and Iowa.[4] A serotype 1 mammalian orthoreovirus (MRV1) was isolated from pigs in Japan in 1984, but no cases have been reported since then.[11]

3.4 Morbidity and Mortality

Experimental infection with the U.S. isolate of MRV3 resulted in 100% mortality in neonatal piglets. The neonates developed acute gastroenteritis and severe diarrhea within three days of inoculation.[4]

The introduction of MRV3 on a farm of over 1,000 pigs in China resulted in respiratory disease in roughly 100 piglets. Enteric disease and deaths of both piglets and adult swine were also reported during the outbreak, although exact numbers are not known.[5]

4. Transmission

In swine, virus replication is known to occur within enterocytes and is shed in the feces of infected pigs.[4] Transmission may be direct or indirect, via contaminated food or water.[12] In general, transmission of orthoreoviruses in mammals occurs mainly by fecal-oral route. Proteolytic enzymes in the mammalian gut can increase infectivity of the virus resulting in systemic infection; lesions may be insignificant or nonspecific.[1] Mammalian orthoreoviruses can also replicate in the respiratory tract and have been detected in nasal secretions[7] and spread by respiratory aerosols.[2]

5. Infection in Swine/Pathogenesis

5.1 Clinical Signs

Mammalian orthoreoviruses have been reported to cause enteritis, pneumonia, encephalitis,[4,13] and reproductive failure in swine.[7] Severe diarrhea and acute gastroenteritis, loss of physical activity, and weight loss were observed in neonatal piglets infected experimentally with the U.S. isolate of porcine MRV3 discovered in 2013. Similarly, orthoreoviruses have been implicated in outbreaks of diarrhea in Chinese and Korean swine.[4] Experimental infection with MRV1 has also been associated with enteritis and pneumonia.[5] Along with fever and inappetence, infected piglets presented with cough, nasal discharge, sneezing, and occasional diarrhea.[11]

5.2 Postmortem Lesions

In earlier cases of experimental inoculation of pigs, only mild microscopic lesions and minimal gross lesions were observed.[7] Recent inoculation with more pathogenic U.S. strains of MRV3 has produced catarrhal enteritis, intussusception, villous blunting and fusion, multifocal necrosis of mucosal epithelium, and vacuolation of intestinal epithelial cells in neonatal pigs. Hepatocellular changes, protein casts in the renal tubules, and suppurative bronchopneumonia were also seen in some.[4]

6. Diagnosis

6.1 Clinical History

The type of disease caused by MRV in swine varies, and infection with the virus does not always result in clinical illness.[7] New porcine strains of MRV3 were discovered in the U.S. in connection with investigations into the outbreak of PEDV which began in 2013. During this time, MRV3 was detected in 37% of the 48 fecal samples taken from neonatal pigs at facilities with PEDV. Co-infection with PEDV was not reported, and no MRV was discovered in 36 samples from healthy pigs at uninfected farms or facilities that had reported past PEDV outbreaks.[4] An earlier epidemiological study in South Korea indicated that porcine orthoreoviruses were endemic in diarrheic swine there, with 19% of samples testing positive for the virus.[10] Isolated cases from individual pigs or farms in China[5,9] and Japan[6] have contributed little to understanding the epidemiology of MRV.

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Complement fixation, immunofluorescence assay (IFA), and immunodiffusion can be used in the detection of a common group antigen shared by all mammalian orthoreoviruses. Mouse monoclonal antibody specifically against the MRV3 σ1 protein has also been utilized for both indirect[4] and direct IFA.[10] Serum neutralization or hemagglutination inhibition (HI) are necessary for serotype identification. Porcine orthoreoviruses are capable of agglutinating swine and human type O erythrocytes.[7]

A wide variety of cells have been used to grow reoviruses.[7] The U.S. strains of porcine MRV3 have been isolated using chicken embryos or baby hamster kidney (BHK-21) cells,[4] while African green monkey kidney (Vero) cells,[9] fetal rhesus monkey kidney cells (TF-104),[10] and swine testicular (ST) cells have been used to isolate Asian strains.[5] Primary porcine kidney (PK) cells were used to isolate MRV1.[11] Presence of MRV3 in swine has been confirmed by reverse transcription polymerase chain reaction (RT-PCR) with primers specific to the S1[4] and L1 gene,[10] and also by nested PCR.[5]

The National Veterinary Services Laboratory (NVSL) in Ames, Iowa currently offers virus isolation of reovirus from swine.[14]

6.3 Tests to Detect Antibody

In laboratory animals, enzyme immunoassay with an MRV3 antigen can be used to detect infection with all the major reovirus serotypes.[1] Indirect IFA and immunoperoxidase monolayer assay (IPMA) have been described for the detection of anti-MRV3 antibodies in pigs,[5] and IFA is also offered at the NVSL.[14]

6.4 Samples

6.4.1 Preferred Samples

The U.S. isolates of MRV3 were detected in feces of diarrheic piglets and ring-dried swine blood meal.[4] Fecal samples,[5,10] as well as spleens, have also been used in identification of Asian strains of porcine MRV.[5] Nasal swabs and tonsils were utilized for isolation of MRV1 in pigs with respiratory disease in Japan.[11] Placentas and fetal tissues from sows inoculated with reovirus between 40 and 85 days of gestation also contain the virus.[7]

Antibody testing of serum can also aid in diagnosis of MRV infection.[5]

6.4.2 Oral Fluids
The use of oral fluids as a diagnostic specimen has not been evaluated for MRV in swine.

7. Immunity

7.1 Post-exposure

Pigs inoculated with MRV can show detectable levels of the virus in feces and nasal secretions as quickly as 24 hours after exposure. Widespread infection among herds is suspected, and antibodies to MRV1, MRV2, and MRV3 have been detected in pigs. Antibodies that inhibit hemagglutination peak at 11-21 days post-infection and may be detected in as few as seven days. Maternal antibodies offer protection for up to 11 weeks, after which time the piglets will be susceptible to infection.[7] In both natural and experimental MRV infection, many young animals appear to be more susceptible than their immunocompetent adult counterparts.[2,4]

7.2 Vaccines

Presently, there are no vaccines available against MRV in swine, other animals, or in humans.

7.3 Cross-protection

There is a group antigen common to all MRVs used for diagnosis;[7] however, antibodies that inhibit viral infectivity and inhibit hemagglutination are serotype-specific.[10]

8. Prevention and Control

Orthoreoviruses are stable across a wide pH range and resistant to lipid solvents.[1] Further, the discovery of chloroform-resistant, thermostable isolates in U.S. swine, especially in ring-dried swine blood meal, is an indicator that existing biosecurity practices may be insufficient to control the spread of enteric disease in swine.[4] Prevention of MRV infection in colonies of laboratory rodents is best achieved with good sanitation, periodic serologic testing, and testing and quarantine of new animals prior to introduction.[1] On-farm movement control, appropriate disposal of dead pigs and slurry, introduction of animals of known health status, and “all-in-all-out” practice can aid in effective control of viral diarrhea outbreaks in swine.[15] These measures, along with an understanding of the characteristics of new pathogenic strains, can aid in the development of new control measures for MRV.

Lithium chloride (LiCl) has shown potential as an antiviral agent against MRV3. After MRV3 was isolated from a single pig with diarrhea, Vero cells were infected with the virus. The early stage of viral replication in Vero cells infected with this strain was inhibited by treatment with LiCl, although further study is required to assess the efficacy of LiCl *in vivo*.[13]

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

The 2016 OIE Terrestrial Animal Health Code does not cover orthoreovirus. There are no recommendations for importation of swine from countries or zones infected with orthoreovirus.

10. Gaps in Preparedness

The discovery of pathogenic isolates of MRV3 in U.S. swine indicates that additional testing and surveillance is required to control outbreaks of enteric disease. The sole focus cannot be swine enteric coronaviruses, and further research is needed to understand the role and extent of involvement of orthoreoviruses in these outbreaks. In a South Korean study, 93% of the samples from diarrheic piglets that were positive for orthoreovirus also tested positive for other enteric pathogens, such as *E. coli*,

Salmonella, or rotaviruses.[10] For this reason, the exact role MRV3 plays in the development of disease and the potential interactions between the various pathogens must be studied in greater detail. Further, the discovery of thermostable MRV3 in ring-dried swine blood meal points to a mode of transmission that may need to be reevaluated, and biosecurity measures in place for PEDV may not be adequately preventing the spread of these novel viruses. Phylogenetic analyses indicate that the MRV3 isolates in the U.S. are reassortant viruses originating from bat, human, and porcine strains.[4] Additional genomic sequencing and epidemiological studies are warranted to determine how the virus arrived here and its true disease potential in U.S. swine herds.

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