

# PORCINE RUBULAVIRUS (BLUE EYE)



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## SUMMARY

### Etiology

- Porcine rubulavirus (PoRV) is an enveloped RNA virus in the genus *Rubulavirus*, family *Paramyxoviridae*.
- There are five subgroups classified based on the hemagglutinin-neuraminidase (HN) gene sequence.

### Cleaning and Disinfection

- Paramyxoviruses are typically labile and easily inactivated by heat and ultraviolet light, as well as low pH levels.
- Paramyxoviruses are susceptible to acids, alcohols, aldehydes, alkalis, halogens, and oxidizing agents, and have limited susceptibility to biguanides, phenolic compounds, and quaternary ammonium compounds.

### Epidemiology

- Swine are the only species that are clinically affected, but antibodies to PoRV have been demonstrated in cats, rabbits, peccaries, mice, and rats.
- PoRV is not known to affect humans.
- Discovered in the 1980s, PoRV is found only in Mexico, where it is considered an important disease affecting swine production. PoRV occurs throughout the year, but most cases are reported between April and July.
- In piglets, morbidity is between 20–50%; mortality can reach 90%.

### Transmission

- Virus is found in nasal secretions, urine, and semen.
- The major route of transmission is direct contact (nose-to-nose). Subclinically infected pigs spread the disease to susceptible pigs.
- Transmission via semen and fomites may also be possible.

### Infection in Swine/Pathogenesis

- In piglets, CNS signs are most commonly observed. The respiratory system can also be affected. Ocular manifestations include blindness, nystagmus, dilated pupils, anterior uveitis, conjunctivitis, swollen eyelids with exudate, and corneal opacity.

- Reproductive signs are seen in pregnant females, including irregular return to estrus, stillbirth, and mummification. Corneal opacity is occasionally noted.
- Boars infected with PoRV may develop signs of anorexia, coughing, orchitis and epididymitis followed by testicular atrophy, conjunctivitis, decreased spermatozoon concentration and motility, and persistent corneal opacity.

### **Diagnosis**

- Virus can be cultured on pig kidney cell lines. Antigens can be detected by direct immunofluorescence in tissue sections and monolayers. There are also quantitative real-time polymerase chain reaction (qRT-PCR) assays that detect either the phosphoprotein gene or the nucleoprotein gene across all strains of PoRV.
- Antibodies can be detected via hemagglutination inhibition, virus neutralization, and enzyme-linked immunosorbent assay (ELISA).

### **Immunity**

- Lifelong immunity typically develops after natural infection with PoRV.
- A killed, oil-adjuvanted vaccine is commercially available in Mexico to prevent PoRV infection.

### **Prevention and Control**

- Modern swine production practices (such as all-in, all-out) can decrease transmission. Standard biosecurity practices will also help prevent PoRV from spreading.
- Disease-free status can be confirmed by serological testing of sentinel animals.

### **Gaps in Preparedness**

- PoRV has never been detected in the United States. There is no PoRV vaccine available for commercial use in the United States.

## OVERVIEW

Porcine rubulavirus (PoRV) is an enveloped RNA virus in the family *Paramyxoviridae*, genus *Rubulavirus*. PoRV emerged in the 1980s, and its distribution appears to be limited to Mexico. PoRV causes a disease commonly referred to as “blue eye disease” because of the corneal opacity seen in some affected pigs.

There are five main antigenic subgroups of PoRV based on the hemagglutinin-neuraminidase gene, which encodes the main protein conferring virulence. Clinical disease has only been observed in swine; however, antibodies have been discovered in cats, rabbits, peccaries, and people presumably following natural infection. Experimental exposure has led to disease in mice, rats, and chick embryos.

Approximately 20% of litters will be affected during an outbreak. Affected litters experience 20–50% morbidity with 90% mortality of affected piglets. Most piglet fatalities will occur within a few days after onset of clinical signs. Morbidity is approximately 2% in pigs over 30 days of age.

PoRV is transmitted via direct contact through infected nasal secretions and urine, and possibly through semen. Spread of PoRV between farms will usually occur through introduction of new, subclinically infected pigs to naïve pigs on the farm. PoRV may be observed year round in Mexico, with most cases occurring between April and July.

Clinical signs in suckling piglets include central nervous system (CNS) signs. Ocular signs can be seen including corneal opacity, blindness, nystagmus, dilated pupils, anterior uveitis, conjunctivitis, and swollen eyelids with exudate. Weaned pigs have fewer CNS signs and mortality, but anorexia, fever, and respiratory signs can be seen. Reproductive signs in pregnant sows and gilts include irregular return to estrus, stillbirth, and mummification. Sows and gilts may also experience anorexia and corneal opacity. Infected boars may exhibit anorexia, coughing, orchitis, and epididymitis. Testicular atrophy (often unilateral) can be seen, along with decreased spermatozoon concentration and motility. Conjunctivitis and corneal opacity also occur.

Virus can be cultured on pig kidney cell lines. Antigens can be detected by direct immunofluorescence in tissue sections and monolayers, and quantitative real-time polymerase chain reaction (qRT-PCR) assays have been developed to detect either the phosphoprotein gene or the nucleoprotein gene across all strains of PoRV. Tests used to detect antibodies include hemagglutination inhibition, virus neutralization, and enzyme-linked immunosorbent assay (ELISA). The immunogenic response to PoRV induces lifelong antibody production. As PoRV lineages diverge, cross-protection across strains may decline in the future. Important samples to collect for PoRV diagnosis in piglets and adults are brain, tonsil, and lung. In pregnant females, PoRV may be isolated from ovary, placenta, and lymph node. Fetal samples collected should include brain, lung, and liver. Nasal and oral swabs along with semen can be used when using qRT-PCR.

Commercially available vaccines in Mexico for PoRV are killed and oil adjuvanted. Vaccination of pregnant females, suckling piglets, and weaned piglets has been demonstrated to provide good immunity against PoRV. Antibody can be found in colostrum of lactating females. Monovalent vaccination against one PoRV isolate may not provide complete cross-protection against other antigenic subtypes.

PoRV should be controlled using standard biosecurity protocols. When replacing pigs, quarantine and serological screening should be used to prevent introduction of PoRV into a naïve facility. To remove PoRV from a facility, control measures include all-in, all-out movement; cleaning/disinfecting; elimination of clinically sick animals; and proper removal of dead pigs. Serological testing of seronegative sentinel pigs can be used to confirm removal of PoRV from a facility.

## LITERATURE REVIEW

### 1. Etiology

#### 1.1 Key Characteristics

Porcine rubulavirus (PoRV) is an enveloped RNA virus classified into the *Rubulavirus* genus under the family *Paramyxoviridae* and subfamily *Paramyxovirinae* and is also known as “blue eye paramyxovirus”.<sup>1</sup> PoRV is believed to have originated from fruit bats in Central and South America. The closest related virus to PoRV is the Mapuera virus which has been isolated from a fruit bat in Brazil.<sup>1</sup>

#### 1.2 Strain Variability

PoRV can be further classified into different isolates and placed into five different subgroups based on the hemagglutinin-neuraminidase (HN) gene sequence.<sup>2</sup> The HN protein confers virulence and alterations to the protein cause changes in virulence.<sup>3</sup>

Subgroup two, containing isolates PAC6–PAC9, can cause increased neurological signs in fattening and adult pigs compared to isolates of the other subgroups.<sup>3</sup> Subgroup one contains the isolates LPMV and PAC4/1993; subgroup two contains the isolates PAC2/1990 and PAC3/1992; subgroup three contains the isolates PAC6/2001, PAC7/2002, PAC8/2002, and PAC9/2003; subgroup four contains the isolates CI/1991, CII/1991, and CIII/1999; and subgroup five contains CIV/1999.<sup>4</sup> Low antigenic relatedness values (ARV) have been found between PAC3/1992 and PAC8/2002, and between PAC3/1992 and PAC9/2003.<sup>2</sup> Low ARVs indicate antigenicity of PoRV beyond the HN gene and inclusion of PAC3/1992 into a serogroup different than PAC8/2002 and PAC9/2003.<sup>2</sup>

### 2. Cleaning and Disinfection

#### 2.1 Survival

Paramyxoviruses are typically labile and easily inactivated by heat and ultraviolet light, as well as low pH levels.

#### 2.2 Disinfection

Paramyxoviruses are susceptible to acids, alcohols, aldehydes, alkalis, halogens, and oxidizing agents, and have limited susceptibility to biguanides, phenolic compounds, and quaternary ammonium compounds.<sup>6</sup>

### 3. Epidemiology

#### 3.1 Species Affected

Clinical signs due to natural exposure have only been seen in swine.<sup>7</sup> Natural exposure leads to antibody production in cats, rabbits, and peccaries.<sup>7</sup> Experimental exposure can cause disease in mice, rats, and chick embryos.<sup>7</sup>

#### 3.2 Zoonotic Potential

PoRV has not been reported to cause clinical symptoms in people.<sup>7</sup> However, swine veterinarians in Mexico have been found to be seropositive to PoRV at a rate of 2.3% and 5.8% using hemagglutination inhibition and virus neutralization assays, respectively.<sup>8</sup>

#### 3.3 Geographic Distribution

PoRV has only been reported in Mexico since its discovery on a farm in La Piedad Michoacán, Mexico in the 1980s.<sup>7</sup> Outbreaks have occurred in 16 of Mexico's 31 states with most of the current outbreaks occurring in central and west-central Mexico.<sup>4</sup> Seroprevalence of PoRV in the Mexican states of Estado

de Mexico, Guanajuato, Jalisco, and Michoacán was found to be between 9% and 23.7% in non-vaccinated swine, making blue eye disease one of the four most important diseases affecting swine in Mexico.<sup>4</sup>

### **3.4 Morbidity and Mortality**

PoRV cases may be observed throughout the year in Mexico, although most occur between April and July.<sup>5</sup>

Approximately 20% of litters are affected during an outbreak.<sup>7</sup> Within these litters, morbidity is 20–50% and mortality of affected piglets is approximately 90%.<sup>7</sup> In the initial stages of an outbreak, suckling piglets usually die within two days of the onset of symptoms.<sup>7</sup> Weeks later in the outbreak, piglets will die within 4–6 days of the onset of symptoms.<sup>7</sup> Depending on the quality of management on a farm, outbreaks can last between 2–9 weeks.<sup>7</sup> In pigs older than 30 days, less than 2% of individuals are affected during an outbreak with an accompanying low mortality rate.<sup>7</sup>

## **4. Transmission**

Virus is found in nasal secretions and urine, along with semen.<sup>7</sup> Direct contact (nose-to-nose) is the major mode of transmission. Spread to new farms usually occurs through introduction of pigs with subclinical infection.<sup>7</sup> Fomites may also contribute to transmission.

## **5. Infection in Swine/Pathogenesis**

Initial replication occurs in the nasal mucosa and tonsil.<sup>7</sup> Soon after, PoRV spreads to the brain via cranial nerves proximal to the oral cavity.<sup>7</sup> Thereafter, PoRV spreads into the lungs and to various organs throughout the body via the blood.<sup>7</sup>

### **5.1 Clinical Signs**

Suckling piglets 2 to 15 days-old are the most profoundly affected.<sup>7</sup> CNS signs and high mortality are observed in this age group.<sup>7</sup> Initial signs include pyrexia, rough hair coat, arching of the back, and GI signs.<sup>7</sup> Soon after, respiratory and neurologic signs may be seen including ataxia, rigidity of the hind limbs, weakness, abnormal sitting positions, and hyperexcitability when handled by people.<sup>7</sup> Possible ocular signs include blindness, nystagmus, dilated pupils, anterior uveitis, conjunctivitis, swollen eyelids with exudate, and corneal opacity.<sup>7</sup>

In weaned pigs, CNS signs and mortality are less common, but other non-neurologic symptoms may be seen including anorexia, fever, and respiratory signs.<sup>7</sup> Atypical outbreaks characterized by increased neurological symptoms and mortality in fattening and adult pigs have been observed.<sup>7</sup>

Reproductive problems including irregular return to estrus, stillbirth, and mummification are likely to be observed in pregnant females.<sup>5</sup> Beyond reproductive failure, clinical signs other than mild anorexia and occasional corneal opacity are not normally observed in adult animals.<sup>12</sup> Boars infected with PoRV may develop signs of anorexia, coughing, orchitis and epididymitis followed by testicular atrophy, conjunctivitis, decreased spermatozoon concentration and motility, and persistent corneal opacity.<sup>11</sup>

### **5.2 Postmortem Lesions**

Piglets frequently develop pneumonia on the ventral tips of the cranial lung lobes.<sup>7</sup> Distension of the urinary bladder and stomach are observed along with fibrinous peritoneal fluid accumulation.<sup>7</sup> An increase in cerebral spinal fluid is common along with cerebral congestion, and ocular lesions such as conjunctivitis, anterior uveitis, and corneal opacity (blue eye) are frequently seen.<sup>7</sup> Vesicles, ulcers, and

exudative anterior uveitis are seen on occasion.<sup>7</sup> Hemorrhage in the thoracic and abdominal cavity may occur, mostly in the pericardium and kidney.<sup>7</sup>

Pregnant gilts may have focal hemorrhaging and congestion on the placenta and endometrium.<sup>12</sup> Fetuses will often be mummified and those not mummified are frequently smaller than normal with areas of dermal ecchymoses.<sup>12</sup>

In boars affected by PoRV, scrotal and tunica vaginalis edema is seen postmortem.<sup>11</sup> The head of the epididymis may have elevated, yellow/white nodules containing brown exudative fluid.<sup>11,13</sup> After inflammation has subsided, boars can be found with testicular atrophy which is commonly unilateral in nature.<sup>13</sup> Histologically, affected boars undergo epithelial degeneration in the head of the epididymis and testicles where most of the inflammation has occurred.<sup>13</sup> In the testicles, a variety of microscopic lesions can be found including seminiferous tubule degeneration, rupture, and vacuolation, mononuclear infiltrate, interstitial cell changes, fibroblast proliferation with fibrosis, and intraluminal multinucleated cells.<sup>13</sup> In the epididymal head, possible lesions encountered are seminiferous tubule rupture, de-epithelialization, spermatozoid phagocytosis, missing spermatid cells, and epididymal head obliteration.<sup>13</sup> Spermatid granulomas are also possible in the head of the epididymis.<sup>11</sup>

## **6. Diagnosis**

### **6.1 Clinical History**

Signs such as encephalitis, corneal opacity, reproductive failure (sows), and orchitis and epididymitis (boars) are suggestive of PoRV.<sup>7</sup>

### **6.2 Tests to Detect Nucleic Acids, Virus, or Antigens**

Virus can be cultured on PK-15 cells or primary pig kidney cells.<sup>7</sup> Separate quantitative real-time PCR (qRT-PCR) methods have been developed to detect the phosphoprotein (P) gene or the nucleoprotein (NP) gene across all known strains of PoRV.<sup>14</sup> Antigen can also be detected by direct immunofluorescence in tissue sections and monolayers.<sup>7</sup>

A qRT-PCR assay to detect the P gene of PoRV was used on brain, lung, and semen samples.<sup>15</sup> PoRV RNA was found using this assay even in pigs which had recovered from an infection more than a year prior to sampling.<sup>15</sup> The specificity was reported to be 100% as the qRT-PCR assay did not amplify any of the RNA from uninfected cells or cells infected with a different RNA virus, and the assay is able to detect all of the known strains of PoRV.<sup>15</sup>

Another qRT-PCR assay has been developed to detect the NP gene of PoRV.<sup>14</sup> The NP protein is well conserved across all genotypes and strains of PoRV, and this detects viral RNA from any strain of PoRV.<sup>14</sup> The detection limit is  $10^2$  RNA copies or a 50% tissue culture infectious dose (TCID<sub>50</sub>) of  $10^2$ .<sup>14</sup> The detection sensitivity from nasal and oral swab samples are 87.1% and 83.9%, respectively.<sup>14</sup> These sensitivities are higher than the sensitivity values for virus isolation of nasal and oral swabs (38.7% and 61.3% respectively), making the qRT-PCR the better choice for detecting PoRV from infected pigs.<sup>14</sup> The specificity for nasal and oral fluid samples are both reported to be 100%, as the qRT-PCR did not produce any false positives from cells infected with other respiratory pathogens.<sup>14</sup>

### **6.3 Tests to Detect Antibody**

A variety of tests can be conducted using paired serum samples collected 14 days apart to detect recent infections. The hemagglutination inhibition (HI) assay is most frequently used but is prone to false-positives.<sup>7</sup> Bovine erythrocytes are preferred to chicken erythrocytes due to the higher rate of false-positive results from chicken erythrocytes.<sup>7</sup> Virus neutralization and ELISA (indirect or blocking) are also

appropriate.<sup>7</sup> A blocking ELISA against the HN protein can offer 99% sensitivity and 97% specificity and could be used for routine screening.<sup>16</sup> Antibodies that develop from natural infection persist for life.<sup>7</sup>

## **6.4 Samples**

Brain is the preferred tissue for virus isolation, however, lung and tonsils are also acceptable.<sup>7</sup> PoRV may be isolated from ovary, placenta, uterus, and lymph nodes in infected pregnant females.<sup>12</sup> Virus may also be isolated from fetal brain, lung, and liver.<sup>12</sup>

## **7. Immunity**

### **7.1 Post-exposure**

After natural infection by PoRV, pigs will develop lifelong humoral immunity to the virus.<sup>17</sup> However, as PoRV lineages deviate, cross-reactivity between different isolates may decline and lead to decreased adaptive immunity.<sup>4</sup>

### **7.2 Vaccines**

Innovac® Ojo Azul®, produced by Avimex Animal Health (Mexico), is a commercial oil adjuvanted, killed MG-55 strain vaccine.<sup>18</sup> Experimental killed, oil-adjuvanted vaccines have been shown to be safe and effective in preventing PoRV infection through vaccination of suckling piglets, weaned piglets, adult pigs, and sows.<sup>19</sup> Sows vaccinated at days 81 and 95 of gestation provided adequate immunity via colostral antibodies to suckling piglets.<sup>19</sup> Information for boar PoRV vaccination was not found. There is no vaccine available for commercial use in the United States.

### **7.3 Cross-protection**

There is incomplete antibody cross-reactivity among different isolates; therefore, monovalent vaccination against one PoRV isolate may not provide adequate cross-protection against other antigenic subtypes.<sup>4</sup>

## **8. Prevention and Control**

PoRV control does not require virus-specific treatments or protocols. When replacing pigs, quarantine and serological testing is used to prevent introduction of PoRV, and standard biosecurity practices are used to prevent infection of a naïve herd.<sup>7</sup> Removing PoRV from a herd requires management practices that include cleaning and disinfecting, closing the herd, all-in-all-out movement of pigs, removing the sickest pigs to prevent shedding and transmission, and proper disposal of carcasses.<sup>7</sup> Confirmation of disease-free status of the herd is done through serologic testing of seronegative sentinel animals.<sup>7</sup>

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

PoRV is not covered in the 2015 OIE Terrestrial Animal Health Code and there are no recommendations on importation of swine or pork.

## **10. Gaps in preparedness**

Because PoRV is endemic to Mexico and has not been seen in the United States, no vaccines have been developed for potential use in the United States.

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