Exogenous Porcine Viruses

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Abstract Porcine organs, cells and tissues provide a viable source of transplants in humans, though there is some concern of public health risk from adaptation of swine infectious agents in humans. Limited information is available on the public health risk of many exogenous swine viruses, and reliable and rapid diagnostic tests are available for only a few of these. The ability of several porcine viruses to cause transplacen-

tal fetal infection (parvoviruses, circoviruses, and arteriviruses), emergence or recognition of several new porcine viruses during the last two decades (porcine circovirus, arterivirus, paramyxoviruses, herpesviruses, and porcine respiratory coronavirus) and the immunosuppressed state of the transplant recipients increases the xenozoonoses risk of humans to porcine viruses through transplantation. Much of this risk can be eliminated with vigilance and sustained monitoring along with a better understanding of pathogenesis and development of better diagnostic tests. In this review we present information on selected exogenous viruses, highlighting their characteristics, pathogenesis of viral infections in swine, methods for their detection, and the potential xenozoonoses risk they present. Emphasis has been given in this review to swine influenza virus, paramyxovirus (Nipah virus, Menagle virus, LaPiedad paramyxovirus, porcine paramyxovirus), arterivirus (porcine reproductive and respiratory syndrome virus) and circovirus as either they represent new swine viruses or present the greatest risk. We have also presented information on porcine parvovirus, Japanese encephalitis virus, encephalomyocarditis virus, herpesviruses (pseudorabies virus, porcine lymphotropic herpesvirus, porcine cytomegalovirus), coronaviruses (TGEV, PRCV, HEV, PEDV) and adenovirus. The potential of swine viruses to infect humans needs to be assessed in vitro and in vivo and rapid and more reliable diagnostic methods need to be developed to assure safe supply of porcine tissues and cells for xenotransplantation.

1 Introduction

Recent interest in the use of porcine organs, cells, and tissues for transplantation in humans (xenotransplantation) to alleviate the critical shortage of human organs has initiated a debate on the possible public health risk from swine infectious agents. Of concern are both the potential adaptation of swine infectious agents to humans and the emergence of new human pathogens (xenozoonoses) (Chapman et al. 1995; Fishman 1994; Weiss 1998; Bach et al. 1998; Gunzburg and Salmons 2000).

The major initial focus of concern has been porcine endogenous retrovirus, because of its potential to integrate into the host genome, its demonstrated ability to infect human cells (Patience et al. 1997; Weiss 1998), and its potential to recombine with human retroviruses. In contrast, limited information is known about the risk to humans of many

exogenous swine viruses, and reliable diagnostic tests are available for only a few of the swine infectious agents that are xenozoonotic risks. It is generally believed that swine can easily be raised free of infectious agents in a sterile environment, and therefore do not pose a significant public health threat. Practical experience has shown this to be a false security, as transplacental infection of fetuses has been demonstrated with a number of viruses, e.g., parvoviruses, circoviruses, and arteriviruses, and contamination of specific pathogen-free (SPF) herds with swine pathogens occurs periodically. Furthermore, a number of new swine viruses, such as porcine circovirus, arterivirus, paramyxoviruses, herpesviruses, and coronaviruses, whose origins are not fully understood, have emerged in the last 20 years. Xenotransplant recipients also are potentially more susceptible hosts for adoption of porcine exogenous viruses as they are immunosuppressed over a long period of time. The greatest risk is from those viruses that have a high mutation rate and/or produce latent or persistent infections. New viruses can also emerge from recombination with human viruses such as herpesviruses and coronaviruses, and reassortment of genes between porcine and human viruses, as demonstrated for influenza viruses.

Exogenous porcine viruses that present the greatest xenozoonoses risk are those that have already been shown to be zoonotic or that infect humans, e.g., orthomyxoviruses, paramyxoviruses, encephalitis viruses, and picornaviruses. Many of these viruses have been extensively studied. Viruses that present unknown yet important potential risk are those that cause persistent and/or latent infections; cross the transplacental barrier and infect swine fetuses; have high recombination or mutation rates; infect cells, organs and tissues used for human transplantation; infect multiple species; infect human or nonhuman primate cells; or cross species barriers. Much of this risk can be eliminated with vigilance and sustained monitoring along with a better understanding of pathogenesis and development of better diagnostic tests.

In this review we present information on selected exogenous viruses, highlighting their characteristics, pathogenesis of viral infections in swine, methods for their detection, and the potential xenozoonoses risk they present. Emphasis has been given in this review to swine influenza virus, paramyxovirus, arterivirus and circovirus as either they represent new swine viruses or present the greatest risk.

2
Orthomyxoviruses

2.1
Swine Influenza Virus

There has been longstanding interest in swine influenza virus (SIV) as a public health risk. The 1918 pandemic of influenza that resulted in 20 million human deaths worldwide has been suspected to be caused by SIV. There has been renewed interest in swine influenza as new strains and types have appeared in swine in the last decade.

2.1.1
Virus Characteristics

Influenza viruses are enveloped, single-stranded RNA viruses belonging to the Family *Orthomyxoviridae*. The viral genome is segmented, with eight segments. Major structural viral proteins are ribonucleoprotein (NP), matrix proteins (M1, M2), hemagglutinin (HA) and neuraminidase (NA). The NP and M proteins are type-specific antigens that are used to identify virus isolates as influenza and to classify influenza viruses as type A, B, or C. Influenza viruses that infect animals are almost exclusively type A viruses, as are most of the influenza viruses of significance in humans. In contrast to the type A viruses, influenza B and C viruses are human viruses and have occasionally been isolated from pigs, probably as limited incidental infections from contact with humans. The more variable HA and NA proteins are used to classify influenza viruses into subtypes. Fifteen HA and nine NA subtypes have been established among type A viruses. Influenza viruses have extensive heterogeneity as a result of reassortment between two viruses, a process known as antigenic shift. They also go through random mutations in the HA and NA, known as antigenic drift, which results in new variants.

2.1.2
Pathogenesis

Swine are quite susceptible to infection with viruses from humans and birds; however, many of the infections are subclinical or result in only mild disease. Serological studies indicate that many such strains remain

in swine populations for only a short time. Occasionally, a human strain will continue to circulate in swine populations long after it had disappeared from human populations (Brown 2000). Until recently, H1N1 and H3N2 had been identified as the predominant subtypes pathogenic for swine. An H1N2 subtype virus, first identified in England in 1995, has become the predominant subtype responsible for clinical disease in England (Brown et al. 1995). H1N2 isolates also have been reported in France (Gourreau et al. 1994), Japan (Ouchi et al. 1996), Belgium (Van Reeth et al. 2000), and the United States (Karasin et al. 2000a). A recent report of H4N6 subtype infection in a single herd in Canada possibly was the first description of this subtype in swine (Karasin et al. 2000b).

The course of influenza virus infection in swine is quite rapid (Easterday and van Reeth 1999). The virus attaches via HA to sialic acid receptors on the surface of epithelial cells lining the airways of the respiratory tract. Virus is internalized and multiplication occurs within the cytoplasm and nucleus with assembly of virions at the plasma membrane. Within 5 h post infection, virus has been observed by electron microscopy budding from the surface of infected cells. Virus initially infects epithelial cells on nasal turbinates and in medium-sized bronchioles in the lung. Infection results in depression and high fever by 24–36 h post infection that lasts for 24–48 h. At 24 h after infection, the bronchiolar epithelium is filled with virus but little damage has occurred. During the next 24–48 h, epithelial cells become necrotic and slough into bronchiolar lumens. Infected cells can be detected at this time in airways of all sizes and in alveoli. The damage to bronchioles stimulates irritation that results in the harsh barking cough characteristic of the disease. Restricted airflow from bronchiolar damage and interference with oxygen transfer due to interstitial pneumonia result in hyperpnea/dyspnea. By 96–120 h after infection, the amount of virus in the lung has dropped considerably, body temperatures have returned to normal, and clinical signs of respiratory distress have abated.

Because of the airborne route of entry and the initial infection of the airways, the gross lesions induced by SIV resemble the bronchopneumonia of bacterial origin. By 48 h post infection, irregularly distributed coalescing gray to red foci of lobular consolidation are evident grossly in the cranial and middle lobes and in hilar areas of the lung (Fig. 1). In severe infections, the entire lung may be edematous and noncollapsed because of interstitial pneumonia. Focal ecchymotic hemorrhages or interlobular bullae may occasionally be present. The consolidated areas

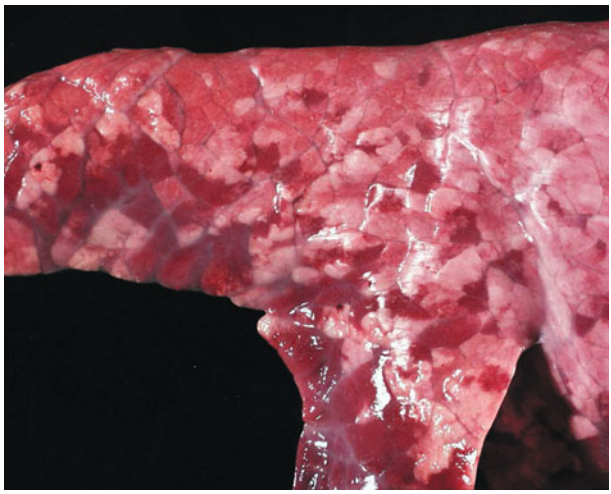

Fig. 1 Lung from a pig infected with SIV. Note *gray* to *red* foci of lobular consolidation

become darker red and depressed below the surface of the surrounding lung as the affected lung tissue contracts during resolution. Gross lesions may remain visible, though shrinking in size, for 2–3 weeks.

Microscopically, the hallmark lesion of influenza virus infection is necrotizing bronchiolitis. By 48 h after infection, necrotic infected epithelial cells are sloughing into bronchiolar lumens and a light loose infiltration of lymphocytes is present around affected airways (Fig. 2). Alveolar walls are thickened by infiltration of mononuclear leukocytes and by pneumocyte swelling. In severe infections, bronchioles and partially collapsed alveoli may contain necrotic cellular debris in which virus can be demonstrated. Alveolar hemorrhage and edema are variable. At 72–96 h after infection, many bronchioles will be lined by attenuated to proliferative epithelium as the airways begin to repair. By 120 h after infection, epithelial proliferation is prominent and little active necrosis is present. Peribronchiolar and perivascular lymphocytic cuffing may be extensive by this time. Lesions vary considerably in severity from lobule to lobule.

Swine influenza is a highly contagious disease. Pigs inoculated experimentally via the intranasal route develop fever and begin shedding virus in nasal secretions within 24 h. Shedding routinely continues for 5–6 days. The primary mode of transmission is through aerosolization of virus in droplets disseminated by the cough induced by virus damage to

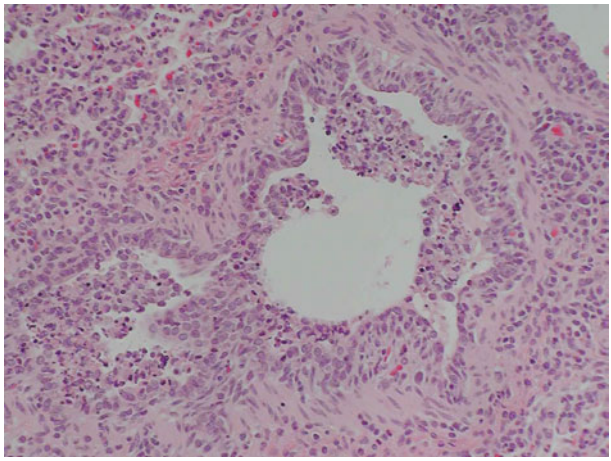

Fig. 2 Photomicrograph of lung from pig infected with SIV showing lesions characterized by necrotizing bronchiolitis, sloughing of necrotic infected epithelial cells into bronchiolar lumens and a loose infiltration of lymphocytes around affected airways

airways in the lung. Infection also can be acquired through direct contact with nasal and oral secretions from infected pigs.

At 24 h after infection, many epithelial cells lining medium-sized bronchioles will contain virus. By 48 h, virus will be present in airways of all sizes as well as in pneumocytes in alveoli. Virus also can be found in epithelial cells lining the nasal passages, but very little virus is present in tracheal epithelium. The tonsil also does not appear to be directly infected. Although a few research studies have reported transient viremia (Brown et al. 1993), in most pigs the virus does not invade beyond the respiratory tract to any great extent.

2.1.3 Diagnosis

A variety of diagnostic tests are available to detect SIV infections (Janke 2000). An antigen-capture enzyme linked immunosorbent assay (ELISA) for type A influenza viruses (Directogen by Becton-Dickinson) developed for use in humans has been used successfully to detect SIV in nasal or bronchiolar swabs. Application of fluorescein-conjugated antibody to frozen sections of lung, and immunohistochemical methods to sections

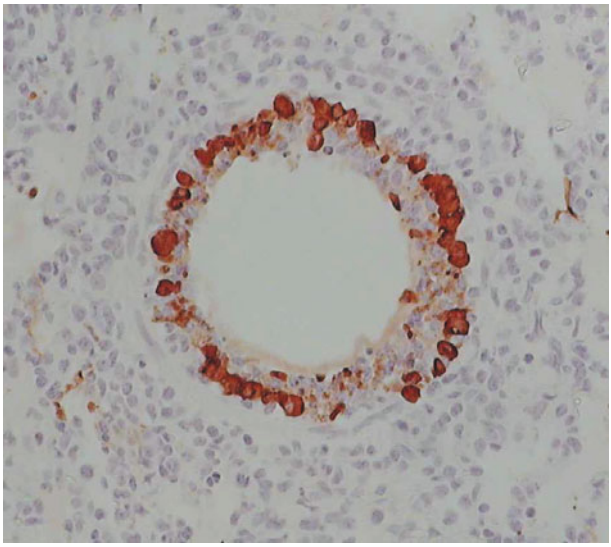

Fig. 3 SIV antigen in lung of a pig infected with SIV detected by immunohistochemistry using monoclonal antibody

of formalin-fixed lung are used routinely to detect SIV in tissues from pigs submitted for necropsy (Vincent et al. 1997; Fig. 3). Virus isolation techniques routinely use embryonated chicken eggs or Madin–Darby Canine kidney cells. Isolates that exhibit hemagglutinating activity are identified as influenza viruses through reaction with hyperimmune typing serum or with polymerase chain reaction (PCR)-based assays. PCR techniques also are used for direct detection of SIV in homogenized lung tissue or on nasal swabs and to determine virus subtype. Hemagglutination inhibition (HI) tests have been the standard serologic test for swine influenza. Recently, a commercially developed ELISA for antibody against H1N1 SIV has become available.

2.1.4
Control and Eradication

SIVs are ubiquitous and very few herds are free of infection. Researchers seeking virus- and antibody-free pigs for experimental purposes have great difficulty locating such herds. Because of this ubiquity and the recurrence of disease each fall in many herds, even without the introduc-

tion of additional pigs that could serve as a source of infection, a carrier state has been proposed. However, no evidence of prolonged infection of individual pigs has been found. Virus probably survives within a herd at a low level by transmission to susceptible individuals within the group. Immune pigs that do not develop clinical illness after challenge have been shown to shed virus in nasal secretions for a short period post infection. Elimination of infection is likely to be possible only by repopulation through segregated early weaning and strict biosecurity measures.

2.1.5
Xenozoonoses

SIVs have been shown to cross the species barrier and infect humans, posing a significant public health risk. Pigs also serve as mixing vessels for generating new strains of influenza viruses through genetic reassortment. SIV subtype H1N1 is suspected to be responsible for 20 million human deaths in the 1918 influenza pandemic. However, the first SIV infection from humans was not demonstrated until 1976 when SIV was isolated from US military recruits (Goldfield et al. 1977). Since then H1N1 has been shown to cross the species barrier and infect humans as SIV was isolated from pigs and caretakers from a farm in Wisconsin (Hinshaw et al. 1978) and from a boy who lived on a farm and spread the virus to classmates. Patriarca et al. (1984) isolated SIV from an animal caretaker who died of pneumonia in Maryland (Wentworth et al. 1994). The latter virus was similar to that circulating in the pig population. Wentworth et al. (1997) reported on infection and mild disease in two individuals conducting experiments in pigs with H1N1 virus. H3N2 of possible swine origin has also been isolated from young children in the Netherlands (Claas et al. 1994).

3
Paramyxovirus

Paramyxoviruses are a diverse group of viruses, some of which present a public health risk. A number of paramyxoviruses of animal origin have clearly crossed the species barrier and have become established as human pathogens; others appear to be narrow in host range. Paramyxoviruses belong to the family *Paramyxoviridae* with four genera: *Paramyxovirus*, *Rubulavirus*, *Morbillivirus*, and *Pneumovirus*. All mem-

bers have a single-stranded negative-sense RNA genome and share affinity for the respiratory tract. In some host species, paramyxoviruses also infect the central nervous system. Members of the genus *Paramyxovirus* include human parainfluenza virus types 1 and 3, and bovine parainfluenza virus type 3. *Rubulavirus* genus includes Newcastle disease virus of birds, Simian virus 5, canine parainfluenza virus type 2, mumps virus, human parainfluenza virus types 2, 4a, and 4b, and Le Piedad paramyxovirus (LPMV) of swine associated with blue eye disease in Mexico. Members of *Morbillivirus* genus include canine distemper virus, measles virus, phocine morbillivirus and rinderpest virus. *Pneumovirus* genus includes human respiratory syncytial virus, bovine respiratory syncytial virus, and turkey rhinotracheitis virus of turkeys. A new genus, *Henipavirus*, to accommodate Hendra virus and Nipah virus, has been proposed. Here we discuss Nipah virus and briefly LPMV and porcine paramyxovirus (PPMV).

3.1 Nipah Virus Infection in Swine

The Nipah virus outbreak was detected in Malaysia in 1999, and resulted in 265 cases of encephalitis in humans, 100 deaths in swine farm workers, and an economic loss of millions of dollars to the swine industry (Tchoyoson-Lim et al. 2000; Parashar et al. 2000). Nipah virus infection was also observed in abattoir workers in Singapore that processed pigs imported from Malaysia (Chew et al. 2000). The disease is characterized by pronounced respiratory and neurologic signs with low morbidity and mortality in pigs. The disease in pigs was closely associated with an epidemic of viral encephalitis in pig farm workers. A viral agent with the morphology of paramyxovirus was isolated and later confirmed to be the agent responsible for the human and swine diseases (Chua et al. 2000a; 2000b). Late presentation of encephalitis was also seen in patients, even months after being exposed to the virus (Wong et al. 2001).

3.1.1 Viral Characteristics

The Nipah virus is an RNA virus with morphology typical of viruses belonging to the family *Paramyxoviridae*. Nipah virus is closely related to Hendra virus of horses (Chua et al. 2000a), formally designated as

equine morbillivirus. Hendra virus was associated with an outbreak of respiratory disease in horses in Hendra, Queensland, Australia, in which 14 horses and one human died (Murray et al. 1995). Comparison of nucleotide sequence of Nipah with other paramyxoviruses shows that Nipah virus is closely related to Hendra Virus but is distinct from members of other genera of the family *Paramyxoviridae* and should be considered a new genus (Harcourt et al. 2000; McCormack 2000). Wang et al. (2000) have suggested *Henipa* as a name for this new genus. Nipah and Hendra viruses have a broad host range that includes humans. The virus replicates in the African green monkey kidney cell line Vero and the baby hamster kidney cell line producing syncytial cells typical of those produced by other paramyxoviruses. Electron microscopy reveals a 'herring bone' appearance of its nucleocapsid, typical of paramyxoviruses.

3.1.2 Pathogenesis

Limited information is available on its pathogenesis and is mostly based on field observations. Clinical disease varies with the age of affected animals and a large number of infected animals have subclinical infection. Adult swine primarily show neurologic signs, but respiratory disease is commonly observed. Mortality was higher in younger pigs of up to 6 months of age. The incubation period is 7–14 days.

The major lesions in lungs include consolidation, emphysema, and petechial to ecchymotic hemorrhages. Mild to severe interstitial pneumonia with widespread hemorrhages and syncytial cells in the endothelial cells of the lung blood vessels are the common histological lesions observed (Hooper et al. 2001). Congestion and edema are common lesions in the brain. Histological lesions in lungs, kidney, and brain tissues include vasculitis with fibrinoid necrosis, hemorrhages, and infiltration of mononuclear cells. Nonsuppurative meningitis with gliosis is common in the brain. High concentrations of viral antigens have been detected by immunohistochemistry in the endothelium of the blood vessels, especially in the lung (Daniels 1999).

3.1.3
Diagnosis

Because of its public health significance, public health officials and the United States Department of Agriculture should be contacted immediately if Nipah virus infection is suspected. All samples should be handled under biosafety level 4 (BL-4) conditions. ELISA and virus neutralization tests are the serological tests currently available. Currently, the viral antigen used in ELISA is gamma-irradiated virus. Before testing, sera are treated with sodium dodecyl sulphate (SDS) and Triton X-100 and by heating at 56 C for 1 h to inactivate virus. Virus can be isolated in Vero cells, which should only be attempted under strict biosecurity conditions.

3.1.4
Control and Eradication

Nipah virus infections have been detected in swine, horses, dogs, and humans. Infections of cats and goats exposed to infected pigs have also been observed. Serological surveys have detected neutralizing antibodies in fruit bats of the genus *Pteropus*, which have been incriminated as a potential reservoir for Nipah virus. Possible modes of transmission between farms within farming communities include movement of infected swine, contaminated boar semen, and dogs and cats. Because of the public health significance of Nipah virus infection, eradication of the disease is the best method of control. In Malaysia, Nipah virus was eliminated by culling all swine from herds affected in Nipah virus outbreaks. Once ELISA became available, swine were tested for serological evidence of Nipah virus infection. Fifteen sows from each of 889 farms were tested of which 50 were found positive. All pigs on these farms were destroyed. This strategy was successful in eradicating Nipah virus infections in Malaysia.

3.1.5
Xenozoonoses

Nipah virus has clearly been shown to infect humans, causing encephalitis with high mortality and hence it is of public health importance. Epidemiologic investigations suggest that pigs are the major source of virus

transmission to humans through direct contact with pigs or their secretions (urine) and excretions (feces) (Goh et al. 2000). Many of the infections occurred amongst pig farmers and abattoir workers (Chew et al. 2000; Goh et al. 2000). Virus may be transmitted by respiratory droplets as persons that did not have direct contact with pigs developed Nipah virus infection (Goh et al. 2000). However, it should be noted that there have been no documented cases of human-to-human transmission of Nipah virus (chan et al. 2002). The main clinical signs in humans include fever, headache, vomiting, reduced level of consciousness, and prominent brain stem dysfunction (Chua et al. 2000a). Fortunately, the virus infection has been eliminated from domestic swine. Fruit bats and other as yet unidentified reservoirs present potential risk for reintroduction of Nipah virus in swine, so constant vigilance is required. Additional studies are needed to determine the reservoirs of Nipah virus and to understand the pathogenesis of Nipah viral infection.

3.2
Menangle Virus

Menangle virus is another porcine paramyxovirus isolated from pigs in Australia (Chant et al. 1998). This virus has been associated with stillbirths in swine (Philbey et al. 1998). Human infections with Menangle virus have been reported. Two workers from a pig farm infected with this new paramyxovirus suffered a respiratory illness and developed high convalescent-phase antibody titers of 128 and 512 to the new virus. Both workers with antibodies had suffered a sudden onset of fever, chills, severe headaches and myalgia. Other personnel with no to low occupational hazard remained seronegative to this new virus. Limited available information suggests that Menangle virus presents a public health and xenozoonoses risk. Information on its epidemiology is limited and additional studies are justified.

3.3
Le Piedad Paramyxovirus

LPMV is a causative agent of blue eye disease first observed in Le Piedad, Mexico (Stephano et al. 1988; Stephano 1999). The virus has not been detected in the USA. LPMV is a member of *Rubulavirus* genus of *Paramyxovirinae* subfamily of *Paramyxoviridae* family. Virus character-

istics as to the morphology, size, and physicochemical properties are typical of other paramyxoviruses; however, LPMV is distinguishable antigenically. LPMV is associated with respiratory, reproductive, and neurological disease in pigs. Clinical signs include fever, arched back, nervous signs of ataxia, weakness, muscle tremor, and abnormal posture. Pigs are hyperexcitable and squeal with paddling movements. Conjunctivitis and corneal opacity are common. Reproductive failure is characterized by return of females to estrus, stillbirths and mummified fetuses.

Mild pneumonia is a common gross lesion observed with microscopic evidence of interstitial pneumonia. Microscopic lesions are also observed in brain and spinal cord. LPMV infection should be suspected in countries where this virus is prevalent when encephalitis, corneal opacity and reproductive failure in sows or orchitis in boars are observed. LPMV can be easily isolated in pig kidney (PK-15) cell line as it induces syncytium characteristic of paramyxoviruses. Viral antigen can be detected in cells by fluorescent antibody tests. Antibodies can be detected by HI, virus neutralization and ELISA.

3.4
Porcine Paramyxovirus

We have reported a neurological and respiratory disease in a swine herd in the northern USA associated with PPMV (Janke et al. 2001). A swine unit with 400 pigs suffered an outbreak of neurological and respiratory disease. Disease spread to second unit within a few days. The clinical respiratory and neurological disease was similar to that observed with PPMV (Nipah virus) infection except the disease was milder. Clinical signs included dyspnea, harsh barking cough, central nervous system (CNS) disturbance, persistent squealing, and whole body tremors, rear end ataxia, and intermittent episodes of head pressing. Gross lesions included congestion of the lungs with consolidation of the ventral tips of the lung lobes. Two pigs were submitted to the veterinary diagnostic laboratory at Iowa State University. Both pigs were in good condition but did exhibit CNS signs as previously described. Clinical signs of respiratory disease were not evident. At necropsy, lungs in both pigs had bilateral pale gray consolidation of the ventral half of the cranial and middle lobes. The rest of the lung tissue was only slightly firmer than normal. No gross lesions were observed in the brain or other internal organs. Mi-

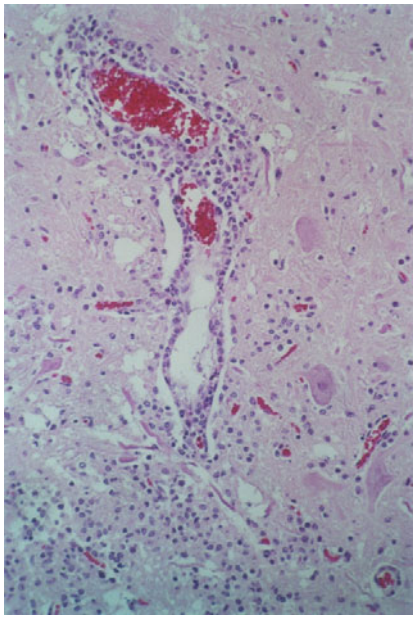

Fig. 4 Lymphocytic perivasculitis in the midbrain of a pig naturally infected with PPMV. Macrophages and lymphocytes surround small vessels. H&E

croscopically, lungs had moderate to severe bronchointerstitial pneumonia characterized by thickening of alveolar septa with infiltrating macrophages and swollen pneumocytes and endothelial cells. Alveolar capillaries were congested. Alveolar lumens were partially collapsed and contained proteinaceous fluid, fibrin, and variable numbers of macrophages, lymphocytes, sloughed pneumocytes, and neutrophils. Epithelium lining of some bronchioles was attenuated and focally disrupted; other bronchioles had intact hyperplastic epithelium. Bronchiolar lumens were filled with neutrophils. Peribronchiolar and perivascular lymphocytic cuffing was prominent. The brain from both pigs had mild but widespread perivasculitis and mild diffuse gliosis (Fig. 4).

Virus was isolated in Vero cells and characterized. PPMV was serologically and genetically related to bovine paramyxovirus 1. The origin of virus has not been identified but based on high sequence homology it is believed to have originated from cattle. There is no evidence as to its public health risk. However, the virus has been shown to replicate in HEp-2 human cell line producing syncytia and can possibly complicate differential diagnosis of Nipah virus infections as there is similarity in

many of the gross and microscopic lesions induced by PPMV and Nipah virus.

Several other paramyxoviruses have been isolated from pigs around the world. These include paramyxovirus isolated from pigs with encephalomyelitis in Japan (Sasahara et al. 1954) and Canada (Greig et al. 1971). Lipkind et al. (1986) isolated paramyxovirus from nasal swabs of swine in Israel. PPMV related to Newcastle disease virus of chickens has been isolated from pigs in Germany (Groschup et al. 1993), and a parainfluenza virus type 2 has been isolated from a sow showing respiratory and reproductive syndrome-like symptoms in Netherlands (Heinen et al. 1998).

4
Arteriviruses

4.1
Porcine Reproductive and Respiratory Syndrome Virus

A new reproductive and respiratory disease characterized by stillborn, mummified and weak-born piglets and pneumonia in neonatal pigs appeared in 1987 in the USA (Hill 1990). The disease was shown to be caused by a new virus designated as porcine reproductive and respiratory syndrome virus (PRRSV) in the USA (Collins et al. 1992) and Lelystad virus (LV) in Europe (Wensvoort et al. 1991). The disease has now become one of the most important viral diseases in swine herds around the world. Salient features of PRRSV infection are persistent infections in swine, in utero infections, and transmission of virus through semen. A high mutation rate leading to high variability and recombination among PRRSV isolates has been reported. Although, no known public health risk has been presented, PRRSV presents a potential xenozoonoses risk due to its high mutation rate, the ability to recombine and the ability to cause persistent infections.

4.1.1
Virus Characteristics

PRRSV is a member of the genus *Arterivirus* under family *Arteriviridae* of the order *Nidovirales*. Other members of this virus family include lactate dehydrogenase-elevating virus of mice, equine arteritis virus, and

simian hemorrhagic fever virus (Plagemann and Moenning 1992). The virions are enveloped and 40–65 nm in diameter, with spikes of 8–12 nm. Six structural proteins have been identified for LV prototype European strain of PRRSV, of which four are glycoproteins. The viral genome is a single-stranded genome of 15 kb with eight open reading frames (ORFs). ORF 1a and 1b code for RNA polymerase and proteases involved in replication, and ORFs 2–7 code for structural proteins. ORF 2–6 products are membrane proteins and the ORF 7 product is a nucleocapsid protein. ORFs 2, 3, and 4 of LV are of 30, 45–50, and 31–35 kDa, respectively. The ORF4 product is believed to contain an important immunogen as monoclonal antibodies to ORF4 have been shown to possess neutralizing activity. ORF7 codes for a nucleoprotein of ~12–15 kDa.

There is extensive antigenic, biological and genetic variability among PRRSV isolates. PRRSV isolates vary in virulence and their ability to cause respiratory disease (Halbur et al. 1995). There also is an extensive variability amongst PRRSV isolates in their ability to cause reproductive failure (Mengeling et al. 1996). Antigenic differences have also been detected between European LV and North American isolates. The American and European strains represent two separate serotypes as amino acid sequence homology in ORFs 2–7 among the US strains is over 90% where it is less than 60% with European strains (Meng et al. 1995). ORF 5 is most variable as amino acid homology amongst PRRSV isolates is 89–92% whereas it is 51–55% between US and European isolates (Meng et al. 1995).

4.1.2 Pathogenesis

The primary route of viral infection is oronasal; however, sexual transmission through semen has been reported. The primary site for PRRSV replication is in macrophages and dendritic cells in tonsils, upper respiratory tract and lungs. Virus replication has been detected in lymph nodes, spleen, thymus, bone marrow, heart and lungs. Viremia is detected 6–12 h after infection and persists for several weeks even in the presence of antibodies.

Clinical disease varies from subclinical infections in pigs on many farms to severe reproductive and respiratory disease on other farms. Virus strain, challenge dosage, and the age and breed of pigs influence the severity of the disease. Clinical disease can vary from subclinical infec-

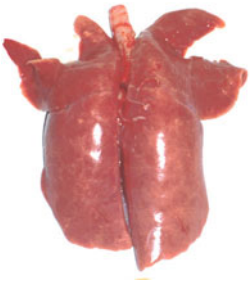

Fig. 5 Lung of a pig infected with highly virulent strain of PRRSV. Note severe consolidation of the lung giving it a rubbery appearance

tion to mild pneumonia for low virulence strains to severe pneumonia for virulent strains. Clinical signs in growing pigs include pyrexia, lethargy, anorexia, dyspnea, tachypnea, chemosis, conjunctivitis, and patchy dermal cyanosis.

Gross lesions can vary from none to pneumonia depending upon the virus strain and the age and genetic strain of the pigs (Halbur et al. 1995). Lungs have mild to severe multifocal involvement and with rubbery appearance and fail to collapse (Fig. 5). Evidence of pneumonia can be seen as early as 3 days after infection and are most severe by 7–10 days and are resolved by 14–28 days. Lymph node enlargement is a consistent gross lesion observed after 10 days of infection. However, microscopic lesions of interstitial pneumonia are common in infected pigs and are characterized by alveolar septal infiltration with mononuclear cells, type 2 pneumocyte hypertrophy and hyperplasia, and alveolar exudates consisting of mixed mononuclear cells and necrotic debris. Lymphohistiocytic encephalitis, myocarditis, and rhinitis may be observed with some strains.

PRRSV replicates in cells of the immune lineage and is believed to cause immunosuppression or at least immune modulation: infected animals are more susceptible to secondary infections. PRRSV infects and destroys pulmonary alveolar macrophages and pulmonary intravascular macrophages and causes decreased function of antigen presenting cells, dendritic cells and macrophages. Reduction in number of alveolar macrophages and circulating monocytes is postulated to be as a direct result of PRRSV-induced apoptosis, which has been detected in mononuclear cells and in the lung and lymphoid tissues of PRRSV-infected cells (Sirinarumitr et al. 1998). PRRSV has also been shown to be immunosuppressive as in utero PRRSV-infected pigs that are born viremic have been

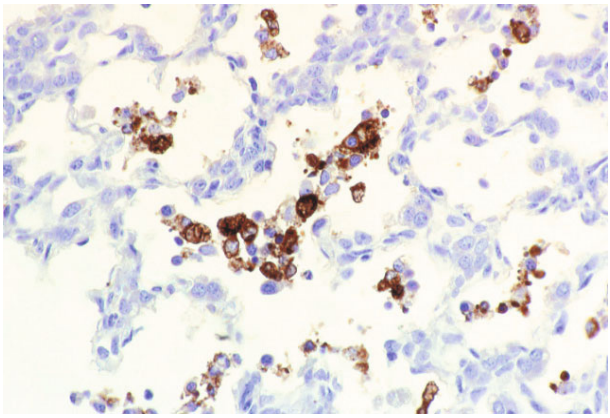

Fig. 6 Immunohistochemical stain of lung from a pig infected with PRRSV. Antigen is seen as a *brown color* within the mononuclear cells

shown to be more susceptible to *Streptococcus suis* infection (Feng et al. 2001).

Antibodies have been shown to be both beneficial and deleterious. Passively acquired antibodies are protective against PRRSV infection. However, antibody dependent enhancement of virus replication has been demonstrated in vitro and is postulated to play a role in PRRSV pathogenesis (Yoon et al. 1996).

4.1.3 Diagnosis

PRRSV can be isolated in primary alveolar macrophage cultures, MA-104 cell lines, and several other proprietary cell lines (Benfield et al. 1992; Meng et al. 1996). Virus is cytolytic for primary alveolar macrophage cultures and macrophage cultures are most susceptible for virus isolation. Viral antigens in cell cultures and tissues can be detected by immunofluorescence (Yoon et al. 1992) and immunohistochemistry (Fig. 6; Halbur et al. 1994). Monoclonal antibodies SDOW 12 and 17 detect a conserved epitope on nucleoprotein of PRRSV and LV (Nelson et al. 1993). These monoclonal antibodies have been very useful in the detection of PRRSV in tissues of infected animals using immunohistochemistry (Halbur et al. 1994). Viral nucleic acid can be detected by in situ hybridization (Haynes et al. 1997) and PCR (Christopher-Hennings

et al. 1995). Virus strains can be differentiated by restriction endonuclease fingerprinting (Wesley et al. 1996). Fluorescent antibody, immunoperoxidase, virus neutralization tests and ELISA are available to detect antibodies. Detectable serum antibodies appear 6–10 days after infection, peak by 4–10 weeks and persist for 5–10 months.

4.1.4
Control and Eradication

PRRSV infections are widespread in swine herds around the world. The source of the virus into the herd is usually new genetic stock. PRRSV is spread either via sexual transmission through semen causing in utero infection of fetuses or through direct contact with pigs that are shedding virus. PRRSV persists in pigs for as long as 157 days (Wills et al. 1997). Virus is shed in feces for over 35 days, urine for 14 days, and saliva for 42 days. Infectious PRRSV has also been detected in semen for 43 days after infection and viral nucleic acid has been detected by PCR in semen for as long as 92 days. A number of management practices such as partial depopulation, segregated early weaning, and multi-site production strategies have been attempted to limit the spread of virus. These methods minimize the duration and severity of the PRRSV-associated disease. However, the best way to control PRRS is to keep PRRSV out of negative herds. This can be accomplished by strict biosecurity practices and assuring that the incoming seed stock is of known PRRSV-free status. PRRSV-free pigs can also be derived by Cesarean section as long as sows are immune to minimize in utero infection of fetuses.

4.1.5
Xenozoonoses

There is no evidence to date of public health risk from PRRSV. Furthermore, we have attempted to infect a large number of human cell lines in vitro with PRRSV but have been unable to show replication. However, additional studies are warranted as PRRSV has been shown to replicate in vitro in nonhuman primate cells. Furthermore, an arterivirus-associated disease, simian hemorrhagic fever, has been observed in monkeys. PRRSV genome is also fairly unstable with a high mutation rate and a vaccine strain has been suspected to have reverted to the wild-type pathogenic strain in Denmark (Botner et al. 1997; Nielsen et al. 2001).

Recombination among PRRSV (van-Vugt-Joke et al. 2001) has also been demonstrated and there is a concern that new strains of PRRSV may emerge through recombination that may adapt in humans through xenotransplantation. Because of these concerns additional studies are warranted to assess xenozoonoses risk of PRRSV.

5
Japanese Encephalomyelitis Virus

Japanese encephalitis (JE) is a mosquito-borne viral disease of animals and humans. In swine, the virus causes lesions in the CNS in young pigs and various forms of reproductive failures in pregnant sows. In other animals, infection is usually subclinical. Geographic distribution of the disease is restricted to Southeast Asia.

5.1
Virus Characteristics

JE virus (JEV) is classified in the genus *Flavivirus* of the family *Flaviviridae*. The virus is spherical, about 40 nm in diameter, enveloped, and it contains single-stranded RNA with an icosahedral capsid. There are three structural and several nonstructural proteins. The structural proteins are envelope glycoprotein E (54 kDa), nonglycosylated envelope protein M (8 kDa), capsid protein C (14 kDa). The oligonucleotide fingerprinting technique (Hori et al. 1986) reveals that mutations of JEV are present in strains that were isolated years apart in Japan and Thailand. JEV is unstable in the environment and easily inactivated by disinfectants. The virus is sensitive to ether, chloroform, and sodium desoxycholate, and also to proteolytic or lipolytic enzymes. It is readily inactivated at 56°C after 30 min and has an optimal pH stability of 8.5. JEV replicates well and produces a cytopathic effect in a wide range of host cell culture systems. Cells originating from mosquitoes also are susceptible.

5.2
Pathogenesis

Pigs become infected with JEV via the bites of mosquitoes carrying the virus. Infected pigs develop viremia that persists for 1–6 days. After the

initial viremia, the virus disseminates to vascular tissues such as the liver, spleen and muscle. The virus enters the CNS via cerebrospinal fluid, endothelial cells, macrophage, lymphocyte infection, or a hematogenous route. In humans and mice, JEV infects and destroys neurons selectively, mostly in areas of the brain stem, thalamus, basal ganglion, and lower layer of cortex. In mosquitoes, JEV infection is noncytopathic.

Transplacental infection of JEV has been reported in pigs (Shimizu et al. 1954). In pregnant pigs, fetuses may become infected during the viremic period. Experimentally, after intravenous infection of pregnant sows with JEV, virus could be recovered from fetuses as early as 7 days post infection. In some pigs, the virus fails to cross the placenta. Successful transplacental infection may depend on the time of gestation at which the dam was infected or on the strain of virus. When infection of pregnant dams takes place in the mid-third of gestation, transplacental infection and pathogenic effects are more obvious. Field observations show that fetal death and mummification have been associated with JEV infection of dams between 40 and 60 days of gestation; fetuses from gilts infected after 85 days gestation were little affected.

No major gross lesions have been found in pigs infected with JEV. However, various abnormalities have been observed in litters from sows infected with the virus during pregnancy. Stillborn or weak neonatal piglets may show hydrocephalus, subcutaneous edema, hydrothorax, ascites, petechial hemorrhages on serous membranes, congestion of lymph nodes, necrotic foci in liver and spleen, and congested meninges or spinal cord. Cerebellar hypoplasia and spinal hypomyelinogenesis have also been described (Morimoto 1969).

Significant microscopic lesions in affected piglets or stillborn pigs have been restricted to the CNS. Most CNS lesions, mainly involving the cortex, basal ganglion, brain stem, and spinal cord, occur in young pigs. Perivascular cuffing and scattered neuronal degeneration and necrosis are found in the cerebrum and cerebellum in pigs. Neuronal degeneration is prominent in the gray matter and Purkinje layers.

Pathologic changes in the testes in association with JEV infection were described (Ogasa et al. 1977). In naturally affected boars, a large amount of mucous fluid was observed in the cavity tunica vaginalis, as well as fibrous thickening along the edge of the epididymis and the visceral layer of the tunica vaginalis. Microscopically, such testicles showed edema and inflammatory changes, with cellular infiltrations in the interstitial

tissue of the epididymis and tunica vaginalis. Cell infiltration and hemorrhage were also evident in the interstitial tissue of the testes.

5.3 Diagnosis

Diagnosis of JEV infection has been based on isolation of the virus from fetuses and infected pigs. Isolation of the virus can be performed by intracerebral inoculation of brain extracts into suckling mice between 1 and 5 days of age. Signs of CNS disturbance or death follow between 4 and 14 days post inoculation, and virus in mouse brain tissue can readily be identified by in vivo neutralization tests in suckling mice or in cell culture. The commonly used cell cultures are derived from hamster, pig kidney, and mosquito, in all of which the virus causes a cytopathic effect. Detection of viral antigen in infected tissues such as brains, placenta and mummified fetuses has also been diagnostically important. Methods such as avidin-biotin staining and fluorescent antibody staining have been used to detect JE viral antigen in formalin-fixed tissues. Several serologic tests are available that detect antibody titers of JEV infection in pig serum such as the HI test, ELISA, antigen biotin-labeled ELISA, single radial hemolysin, and serum neutralization technique. An ELISA to detect IgM antibodies to JEV has been developed (Burke et al. 1985). Detection of cerebral spinal fluid IgM using ELISA diagnostic kits has been used in humans. However, in an area where vaccination is routine, paired serum samples should be considered in the serology. The presence of the antibody in fetuses is of diagnostic value.

5.4 Control and Eradication

Infection with JEV in nature is maintained cyclically, involving vector mosquitoes (*Culex tritaeniorhynchus*), birds, and mammals. A correlation between infection in pigs and infection in humans is apparent, with evidence indicating that swine play an important role in the build-up of the virus within a population. In enzootic zones, pigs are favored feeding sources for mosquitoes. Consistent development of viremia in susceptible pigs ensures a continued supply of infected mosquitoes. However, epidemiologic patterns could differ among areas of Southeast Asia, as activity for the vector mosquito was modified by differing climatic con-

ditions (Takashima et al. 1988). Other animals and different mosquito species could alter infection cycles and have been factors involved in the transmission of JEV. Chickens and wild birds, especially herons and egrets, have been able to maintain the virus.

Population density of susceptible animals for JEV is the most important predisposing factor. In countries within the temperate zone, the mosquito season starts in late spring and the pig–mosquito cycle becomes evident shortly afterward. During this time the pig population contains a high proportion of susceptible breeding pigs, whose passive immunity has waned during the winter. Given these conditions, high concentration of the virus can be built up and maintained in the pig population. This buildup will not be as prevalent in the tropical countries of Southeast Asia, where the mosquito–pig cycle may continue throughout the year.

The treatment of JE in humans with human recombinant interferon-α has shown some satisfactory results. For swine, breaking the infectious cycle in this mosquito-borne disease is an important step. However, because insect control is impractical, immunizing breeding pigs with JEV vaccine is widely applied as a control and preventive measure. Live-attenuated vaccines have been developed for the prevention of JE in pigs and are being used successfully in the field (Hsu et al. 1972; Fujisaki et al. 1975). Inactivated vaccines have proved to be less efficient. It is recommended that attenuated vaccines be given to young gilts or boars twice at an interval of 2–3 weeks before the start of the mosquito season. The live virus vaccines are not recommended for use in pregnant pigs.

5.5 Xenozoonoses

JE is one of the common mosquito-borne diseases of the human CNS, with epidemics having been recorded in several Southeast Asian countries. Pigs are considered the most important natural amplifying animal for the virus. Episodes of human infection occur annually during the mosquito season. Disease due to JEV in most people is subclinical or mild, but fatal encephalitis develops in some children. It should be recognized that tissue suspected of being infected with JEV must be handled with care, because the virus is heat labile and pathogenic to humans. No report is available on JEV infection in humans from pork. Survival of the virus in the environment is not expected because it is highly

temperature sensitive, but human infection can occur during the mosquito season. Inactivated vaccines are available for humans, and are highly recommended for susceptible populations.

6
Encephalomyocarditis Virus

Encephalomyocarditis virus (EMCV) naturally infects rodents and a wide range of vertebrate species. The host range includes chimpanzees, monkeys, elephants, lions, squirrels, mongooses, raccoons and pigs. Rats and mice are believed to be the principal reservoir of the virus. Many rodents are susceptible to experimental infection, showing high levels of virus in their tissues, and infected rodents excrete virus in their feces and urine. The virus has been isolated from dried feces and from intestines of rats or mice captured on farms where swine disease had previously occurred (Gainer 1967; Acland and Littlejohns 1975). Although the virus has been isolated from mosquitoes caught in Africa, Brazil and USA, and from ticks in India, there is no evidence that natural EMCV infection in swine is vector-borne.

Swine is the domestic animal most susceptible to clinical disease by EMCV infection. EMCV infection in pigs was first reported in Panama in 1960, and subsequently in Florida between 1960 and 1966, and in Australia in 1970. The virus causes high mortality in young pigs and reproductive failures in breeding females. Sudden deaths of young pigs due to myocardial failure are common on infected farms, and myocarditis and encephalitis are evident in affected piglets. Clinical problems in swine are mostly limited to tropical areas, and endemic infection with significant economic losses has been identified in certain areas of tropical countries. The disease also has been observed in different zoo animals.

6.1
Virus Characteristics

The virus is classified as genus *Cardiovirus o*f the family *Picornaviridae*. Many properties of EMCV are shared by other picornaviruses. The virus is ether-resistant and stable over a wide pH range. It is inactivated at 60°C for 30 min, but some strains have shown a marked thermal stability. EMCV replicates well in cell cultures originating from several animal species. The virus also replicates in mice and chicken embryos and is

pathogenic to different laboratory animal hosts. EMCV has hemagglutinating ability with erythrocytes of guinea pig, rat, horse and sheep, and most strains require KCl–borate buffered solution for an optimal hemagglutination reaction. The EMCV virions contain a single strand of RNA of molecular weight 2.6×10^6 Da, which comprises 31% of the virion mass and is enclosed in a protein capsid shell. The viral proteins show four non-identical polypeptide bands on SDS–polyacrylamide gel electrophoresis.

6.2 Pathogenesis

The natural infection of swine most probably occurs by the oral route. Following experimental oral infection in piglets, viremia was demonstrated as early as 2 days post inoculation (dpi) and persisted for 2– days. Virus was present in the feces for as long as 9 days following oral administration. The highest virus titers were recovered from heart tissues. Liver, pancreas and kidney contained virus, usually at a greater concentration than blood. Animals that survived acute disease produced anti-EMCV antibodies. The course of infection in swine appears to be influenced by virus strain, viral dose, history and level of viral passage and susceptibility of the individual animal.

Following intramuscular infection of pregnant sows, a transplacental infection with fetal deaths was observed (Love and Grewal 1986). Infected and dead fetuses showed myocardial lesions varying from multiple small foci to large diffuse patches. Some difficulty in producing experimental reproductive disease in pregnant sows was observed with a US isolate. However, transplacental infection was successful when the virus was passaged in young pigs, rather than cell culture, before inoculation (Christianson et al. 1992). Fetal deaths following infection in sows appear to occur as early as 2 weeks post infection. It is not known whether all EMCV strains can cause both the typical myocarditis observed in young pigs and reproductive failure in breeding pigs. Pathogenic variability in swine fetuses by different EMCV isolates has been reported (Kim et al. 1989).

Young pigs dying in the acute phase may show no gross lesions, but myocardial lesions are common in infected pigs. The heart is usually enlarged, with visible yellowish or white necrotic foci (2–15 mm in diameter). The lesions are most commonly observed on the epicardium of the

right ventricle. Infected fetuses become mummified in various sizes depending on the stage of infection and may be hemorrhagic, edematous or apparently normal. The myocardial lesions may be seen in some infected fetuses but it would be difficult to observe under field conditions.

Histopathologically, the most significant finding in young pigs is myocarditis with focal or diffuse accumulation of mononuclear cells, vascular congestion, edema and degeneration of the myocardial fibers with necrosis. Mineralization of necrotic heart muscle is common. Congestion with meningitis, perivascular infiltration with mononuclear cells and some neuronal degeneration may be observed in the brain. Nonsuppurative encephalitis and myocarditis were also observed in swine fetuses with natural EMCV infection.

6.3 Diagnosis

High neonatal mortality with or without a history of reproductive failure is a useful tip in the diagnosis. Dyspnea manifested as rapid abdominal breathing due to heart failure may be observed in young infected pigs. Gross lesions of white necrotic areas in the heart muscle are characteristic of EMCV infection, although such lesions also resemble those caused by vitamin E and selenium deficiency.

A definitive diagnosis should be based on virus isolation, for which heart is the best tissue. Baby hamster kidney (BHK-21), HeLa or Vero cell lines are commonly used for virus isolation. Virus isolation is usually successful from pigs during the acute phase. Microscopically, myocarditis of varying stages with infiltration of mononuclear cells along with nonsuppurative encephalitis is indicative of EMCV infection. Viral nucleic acid can be detected by nucleic acid probes (Meng et al. 1993). Detection of antibody specific to EMCV from stillborn pigs is particularly significant for fetal infection (Joo et al. 1988; Kim et al. 1989).

6.4 Control and Eradication

An important source for swine infection appears to be feed or water contaminated with virus by rats, other rodents or diseased carcasses. The mode of virus transmission is not clear, but rodent-to-pig transmission is probably common. Several outbreaks in Australia were found to

be closely associated with rat and mouse plagues (Acland and Littlejohns 1975). Pig-to-pig transmission has been questioned because sentinel pigs failed to become infected after contact with experimentally infected and sick pigs (Littlejohns and Acland 1975). However, the role of the infected pigs in natural transmission, either directly or indirectly, cannot be excluded, since infected pigs have been shown to excrete the virus at least for a short period.

An inactivated vaccine for EMCV infection in swine is commercially available. The vaccine appears to be effective, since high humoral immunity is detected in vaccinated pigs. On pig farms, it is important to control rodents or minimize their contact with pigs either directly or indirectly via contamination of feed or water. The virus can be inactivated in water using common disinfectants.

6.5
Xenozoonoses

Evidence for human infection with EMCV has been demonstrated by detection of the specific antibodies in human populations (Tesh 1978). However, there is no report that EMCV causes human heart disease.

7
Porcine Circovirus

Porcine circovirus (PCV) was first isolated in 1974 as a contaminant of a porcine kidney cell line (Tischer et al. 1974) and today has emerged as one of the major pathogens of swine. It is ubiquitous in swine populations around the world and is associated with a debilitating disease referred to as post weaning multisystemic wasting syndrome (PMWS). PCV has also been incriminated in porcine dermatitis nephropathy syndrome (PDNS) and in congenital tremors in swine.

7.1
Viral Characteristics

PCV is a member of the genus *Circovirus* in the family *Circoviridae*. The virus is characterized by its nonenveloped, icosahedral particle and a unique circular single-stranded genomic DNA. Other members of this genus include beak and feather disease virus, and possibly pigeon cir-

covirus and goose circovirus. A second genus, *Gyrovirus,* is comprised of one member, chicken anemia virus. A circovirus-like virus, designated as TT virus, has been identified in humans (Miyata et al. 1999). PCV is 17 nm in diameter as detected under the electron microscope. PCV has been divided into two types based on antigenic and genetic differences. The cell culture contaminant PCV-PK-15 has been designated as PCV type 1 (PCV1) and is avirulent. The virulent PCV associated with PMWS has been referred to as PCV2. The genome of the two PCV types is similar and consists of a 1,700 nucleotide single-stranded circular DNA. Homology among isolates within the same type is 95%–99%, while it is less than 80% among viruses of different types (Fenaux et al. 2000). The viral genome has two major open reading frames (ORFs) located in opposite orientations: ORF1 is involved in the replication of the virus genome and is relatively conserved among both types of PCV with 83% nucleotide homology between two types. The ORF2 is much more variable between the two types, with 67% nucleotide homology between two types (Morozov et al. 1998). One major structural protein encoded by ORF2 of 36 kDa in PCV-1 (Tischer et al. 1982) and 30 kDa in PCV-2 was identified (Nawagitgul et al. 2000).

The virion is highly resistant to a pH as low as 3.0 and chloroform treatment, and it is stable after exposure at 56°C and 70°C for a minimum of 15 min (Allan et al. 1994). PCV can replicate in both primary cells and continuous cell lines derived from pig tissues, as well as in Vero cells (Allan et al. 1994). It is commonly propagated in PK-15 cells. For replication, PCV requires cellular enzymes expressed during the S phase of the cell cycle. Replication of PCV is enhanced by treatment of infected cell cultures with 300 mM d-glucosamine for 40–60 min at 4–6 h post infection (Tischer et al. 1987). PCV lacks hemagglutination activity. The in vitro effectiveness of various disinfectants has been reported (Royer et al. 2001).

7.2 Pathogenesis

PMWS is characterized clinically by progressive dyspnea and emaciation in 6–18-week-old pigs. Histologic lesions consist of lymphohistiocytic to granulomatous inflammatory cell infiltrates in multiple tissues, most frequently in the lung, liver, kidney, and lymphoid organs (Fig. 7) (Harding and Clark 1997; Sorden 2000). The most characteristic lesion is deple-

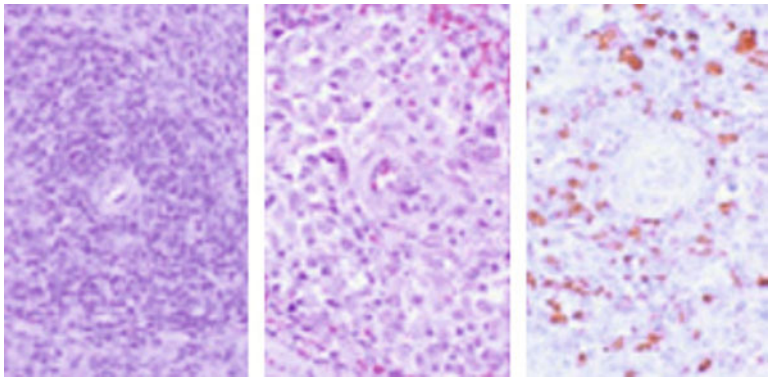

Fig. 7 Sections of spleen from a normal (*left*) and that from a PCV-infected pig with PMWS (*middle* and *right*). Sections were stained with H&E (*left* and *middle*) and or by immunohistochemistry (*right*). Note periarteriolar lymphoid sheath is present in normal porcine spleen (*left*) where as periarteriolar lymphocytes are replaced by macrophages in spleen of a pig with PMWS (*middle*). PCV2 antigen (*brown color*) is abundant in periarteriolar macrophages in pig with PMWS

tion of lymphoid tissues; in some cases, these tissues contain macrophages with intracytoplasmic clusters of basophilic inclusion bodies that ultrastructurally correspond to arrays of PCV particles (Kennedy et al. 2000). PCV2 antigen and nucleic acid are consistently demonstrable in lesions of pigs with PMWS by immunohistochemistry (Sorden et al. 1999) and in situ hybridization (Sirinarumitr et al. 2000).

Results of experimental inoculation of pigs with PCV2 have been highly variable. Gnotobiotic and less than 4-week-old colostrum-deprived pigs have in general developed more severe lesions and clinical disease than conventional pigs. For example, Bolin et al. (2001) inoculated 20–25- day-old Caesarian-delivered, colostrum-deprived (CDCD) pigs intranasally and subcutaneously with a Canadian PCV2 isolate and reproduced severe clinical disease and most of the characteristic gross and microscopic lesions associated with PMWS. This group also demonstrated PCV2 DNA in tissues from experimentally infected pigs at 125 days post inoculation, confirming persistent infection by PCV2. Inoculation of SPF or conventional pigs has induced minimal clinical signs and only mild to moderate lesions. Recently (Fenaux et al. 2002) 4-week-old SPF pigs were inoculated with cloned PCV2 plasmid DNA. Pigs developed mild respiratory disease, but wasting was not observed during

the 35 day duration of the study; gross and microscopic lesions in lymphoid tissues and lungs were consistent with PMWS.

Harms et al. (2001) experimentally inoculated 3-week-old CDCD piglets with PCV2, and the antigens were detected in tissues by immunohistochemistry as early as 7 dpi. High numbers of PCV2 positive cells were found in all tested organs of all pigs from 14 to 28 dpi. The amount of PCV2 antigens in lymphoid organs and tissues was proportionate to the degree of lymphoid depletion. PCV2 DNA was detected in serum, urine, and nasal and rectal swabs from 7 to 49 dpi. Antibodies to PCV2 were first detected at 21–28 dpi.

Experimental inoculation of 1-day-old colostrum-deprived piglets (Allan et al. 2000) and weaned SPF pigs (Magar et al. 2000) demonstrated large amounts of PCV2 antigens in various tissues around 20–26 dpi. Immunofluorescent assay of frozen tissue sections demonstrated PCV2 in trachea, lung, thymus, spleen, lymph nodes, salivary gland, liver, kidney, adrenal gland, pancreas, cecum, colon and small intestine (Allan et al. 2000). PCV2 antigens were detected predominantly in the cytoplasm of cells of monocyte/macrophage lineage of most organs and with lower frequency in the nucleus of macrophages and epithelial cells (Harms et al. 2001; Magar et al. 2000). However, labeling of hepatocyte nuclei was common.

The oronasal route is believed to be the portal of entry for PCV2. In experimentally inoculated pigs (Allan et al. 1995; Harms et al. 2001; Krakowka et al. 2000; Magar et al. 2000), PCV2 antigen and/or nucleic acid are detectable in a wide variety of tissues, including lung, liver, kidney, and intestinal tract, but PCV2 is most consistently found in lymphoid tissues, including tonsil, lymph nodes, spleen, and Payer's patch (Harms et al. 2001). The predominant cell type containing PCV2 in tissues of experimentally- and naturally-infected pigs is the monocyte/macrophage. PCV2 antigen and nucleic acid have also been detected in lymphocytes and a variety of epithelial cells. Antigens of PCV2 have been detected within bronchial and bronchiolar epithelial cells as well as enterocytes lining the tip of intestinal villi by immunohistochemistry, suggesting that these cell types are sites of replication and possibly the ports of entry of the virus.

PCV2-induced PMWS is exacerbated by viral co-infections and immune stimulation. In one experiment, lymphoid tissue homogenates from pigs with PMWS that induced PMWS in gnotobiotic swine were found to contain porcine parvovirus (PPV). Subsequent studies demon-

strated that co-infected pigs developed more severe clinical disease and lesions. Synergism between PCV2 and PRRSV has also been demonstrated: among 3- week-old colostrum-deprived pigs, there was moderate respiratory disease but no mortality in pigs inoculated with PRRSV, lethargy, sporadic icterus and 26% mortality in pigs inoculated with PCV2 alone, and severe lethargy and respiratory disease and 100% mortality in pigs inoculated with both PRRSV and PCV2 (Harms et al. 2001). These experimental findings are highly consistent with what is observed in field cases of PMWS; both PRRSV and PCV2 infection are demonstrated in over half of the cases of PMWS diagnosed at the Iowa State University Veterinary Diagnostic Laboratory. There is also evidence that immunization with an irrelevant antigen may potentiate the development of PWMS. Recently, Krakowka et al. (2001) inoculated gnotobiotic pigs with PCV2 and injected them with keyhole limpet hemocyanin in Freund's incomplete adjuvant. Immunized pigs developed moderate to severe PMWS whereas none of the PCV2-infected but nonimmunized pigs developed PMWS.

PCV2 has been associated with PDNS characterized by multifocal cutaneous infarction, necrotizing (leukocytoclastic) vasculitis in dermal, subcutaneous, renal, and other blood vessels, and fibrinous/necrotizing glomerulonephritis. Most affected pigs are infected with PCV2, and many have lymphoid depletion and other lesions characteristic of PMWS. While necrotizing vasculitis and glomerulonephritis are consistent with type III hypersensitivity response (mediated by deposition of immune complexes within vessel walls), this has not been proven to be the mechanism underlying PDNS (Allan and Ellis, 2000).

PCV has also been incriminated in congenital tremors, a CNS disease that is characterized by myelin deficiency and whole body tremors that appear shortly after birth. Severity of tremors varies from severe to mild and piglets die of starvation as they are unable to suckle. The disease has been reproduced by inoculation of pregnant sows with cell-free brain extracts from pigs with congenital tremors as well as cell culture propagated virus. All samples tested were PCV2 positive. Recently, in situ hybridization demonstrated PCV2 in neurons in the brains of pigs with congenital tremors (Stevenson et al. 2001).

Recently, PCV2 was shown to be vertically transmitted and involved reproductive failure. In 1999, a new sow facility in Canada was confronted with late-term abortion and farrowing of both stillborn and mummified piglets (West et al. 1999). Autopsy examination of a fetus showed

that the ventricles of the heart were dilated, the liver was enlarged and firm, and hydrothorax and ascites were observed. Microscopic lesions included myocardial degeneration with mild fibrosis and diffuse infiltration of lymphocytes and macrophages. Copious amounts of PCV2 antigens were detected in both the nucleus and cytoplasm of cells throughout the myocardium. Livers of multiple fetuses contained PCV2 antigens, primarily in the sinusoid endothelial and Kupffer cells. There are other reports of PCV2-associated reproductive failure (Josephson and Charbonneau 2001). Experimental inoculation studies support the heart as a primary site of PCV2 replication in fetuses. Both PCV1 and PCV2 have been detected in boar semen, supporting the possible role of PCV2 in reproductive disorders (Larochelle et al. 2000).

7.3 Diagnosis

PCV associated PMWS should be suspected if clinical signs of wasting are observed along with histological lesions of lymphoid depletion and/or lymphohistiocytic to granulomatous inflammation in any organs. Presence of viral antigen or nucleic acids confirms the role of PCV. Viral antigens can be detected by Immunofluorescent assay in frozen sections (Allan et al. 1999, 2000; Kennedy et al. 2000) or by immunohistochemistry (Fig. 7) (Sorden et al. 1999) in formalin-fixed, paraffin-embedded tissues. An in situ hybridization technique has been developed to detect PCV2 nucleic acids in histological lesions (Sirinarumitr et al. 2000). PCV2 nucleic acids can also be detected by PCR: several PCR applications have been reported for the detection of PCV or for differentiating PCV types 1 and 2 or typing of PCV, including standard PCR (Larochelle et al. 1999), multiplex PCR for typing PCV1 and PCV2 (Larochelle et al. 1999) combination of PCR and restriction fragment length polymorphism (Hamel et al. 1998), quantitative and competitive PCR for the detection of PCV DNA in serum (Liu et al. 2000), PCR to detect PCV2 in formalin-fixed, paraffin-embedded tissues (Kim et al. 2001) and nested PCR (Larochelle et al. 2000) or combined multiplex and nested PCR for the detection of PCV2 in semen (Kim et al. 2001).

PCV is isolated by inoculating primary porcine kidney cell cultures or the porcine kidney cell line PK-15 (lines which are free of contaminating PCV). Virus replicates in nucleus and viral antigen can be observed in cytoplasm and nucleus by immunofluorescence.

The most commonly used methods to detect PCV2 antibodies are Immunofluorescent and immunoperoxidase monolayer assays (Allan et al. 1999; Dulac and Afshar 1989; Ellis et al. 1998). A recombinant ORF2-based indirect ELISA (Nawagitgul et al. 2002), and synthetic peptide-based ELISA (Walker et al. 2000), which are as sensitive and specific, have been developed.

7.4 Control and Eradication

PCV is ubiquitous in swine herds around the world. It is excreted in oronasal secretions and feces, which serve as the main source of swine infections. In utero infection of swine fetuses is also common as PCV infection has been detected in newborn pigs as well as CDCD pigs. Boars also serve as a source of PCV infection. Passively acquired antibodies are protective and PCV-free pigs have been obtained from PCV-immune sows by isolating them away from their mothers during the first 4 weeks of life and raising them in a PCV-free environment.

7.5 Xenozoonoses

Mixed results have been reported as to the evidence for PCV infections in humans. Two groups of researchers did not detect any antibodies in serum samples from the general human population (Allan et al. 2000) nor did veterinarians working with PMWS affected pigs (Ellis et al. 2000). In contrast, Tischer et al. (1995) found antibodies to PCV in 30.2% of samples from hospitalized patients with fever of unknown etiology. PCV has also been shown to infect human cell lines with limited to no replication (Allan et al. 1994). We have attempted to infect several established human cell lines with PCV2 unsuccessfully. We believe that PCV2 should be considered a potential, although a low, risk as it has been shown to produce persistent infections, to be vertically transmitted and some variability has been discovered. Furthermore, a circovirus-like virus (TT Virus) has been detected in humans, the significance of which is not known.

8
Herpesviruses

Three types of herpesviruses are prevalent in swine: Pseudorabies rabies virus (PRV), also known as Aujeskey's disease virus, porcine cytomegalovirus, and porcine lymphotropic herpesvirus. Of these PRV has been most studied but all three viruses are common in swine and cause persistent infections and represent a risk of xenozoonoses.

8.1
Pseudorabies Virus

Pseudorabies virus infections are common in swine around the world. The salient features of pseudorabies virus are its ability to cause latent infection in sensory ganglia of the nervous system and in lymphoid tissue of the tonsils. Pigs are the only natural host; however, pseudorabies virus infects cattle, sheep, goats, and cats, producing neurological clinical disease that is often fatal (Kluge et al. 1992).

8.1.1
Virus Characteristics

Pseudorabies virus is a member of the *Alphaherpesvirinae* subfamily under the family *Herpesviridae*. Virions are enveloped, 150–180 nm in diameter, and contain a 150-kb double-stranded DNA genome. The virus is well conserved with a single serotype; however, genetic differences have been observed (Paul et al. 1982). Major viral envelope proteins are gB, gC, gD, gE, gI, and gG. Virus is very labile and is sensitive to pH, temperature and common disinfectants.

8.1.2
Pathogenesis

In pigs, the outcome of PRV infection varies from subclinical infection to severe respiratory disease, neurological disease, and/or abortions depending upon the strain of PRV, the challenge dose, and the age of the pigs at the time of infection. Neonatal seronegative pigs are most susceptible and suffer from severe respiratory disease with high mortality. Pigs show clinical signs of respiratory disease and CNS signs, possibly with

respiratory distress. Nursery and grow-finish pigs have fever and anorexia. Infected pigs may exhibit sneezing, coughing, and nasal discharge. In adult pigs, infections are usually subclinical with inappetance and transient fever. Abortions are common in pregnant swine. Gross lesions include none to fibrinonecrotic rhinitis, laryngitis, tracheitis, swollen and hemorrhagic lymph nodes of the upper respiratory tract, and mild keratoconjunctivitis. Microscopic lesions include pulmonary congestion with multiple foci of necrosis and hemorrhage involving septa and airways. Eosinophilic intranuclear inclusion bodies in focal necrotic areas may be detected. Maternal antibodies are protective; however, high doses of virus can overcome low maternal antibodies.

8.1.3 Diagnosis

Pseudorabies should be suspected in neonatal pigs with respiratory disease or in cases of abortions in sows. Microscopic evidence of viral encephalitis along with multifocal necrotizing pneumonia is further suggestive of PRV infection. Confirmation of the presence of PRV antigen by fluorescent antibody tests in frozen sections of tonsils, brainstem, lung or trachea can be used to confirm pseudorabies. PRV infection may also be confirmed by isolation of virus from brain, spleen or lungs. Viral isolates can be differentiated by restriction endonuclease analysis (Paul et al. 1982). Serologic evidence for PRV infection can be obtained by virus neutralization or ELISA. Differential ELISA for the differentiation of vaccinated from infected animals are commercially available.

8.1.4 Eradication and Control

PRV is shed in oronasal secretions. Virus transmission occurs via air, water, and contaminated fomites and infected pigs. The virus causes latent infections, which can be reactivated by stress. The ability of PRV to cause latent infection complicates its eradication and control. Efforts are underway in the USA and a number of other countries to eradicate pseudorabies virus. The availability of gene-deleted vaccines and companion diagnostic tests to differentiate vaccinated from infected animals have made eradication of pseudorabies a possibility. The approaches used for

eradication include vaccination of pigs with gene-deleted vaccines and culling of infected animals.

8.1.5
Xenozoonoses

PRV virus has a broad host range and infects pigs, cattle, sheep, goats, cats and horses. Wild animals including raccoon, opossum, rat, and mouse also are susceptible. Nonhuman primates, such as chimpanzees and apes, are resistant to PRV infection whereas marmosets and rhesus monkeys are susceptible. Limited human infection studies have been conducted. In 1987, serologic evidence for PRV infection was observed in three humans with low level seroconversion after exposure to cats. Anusz et al. (1992) described six of seven workers in contact with cattle developing pruritus on their hands, which spread to their shoulders and backs. Herpesviruses also have been shown to recombine (Umene et al. 1999). Although unlikely, the recombination of swine herpesviruses with human herpesviruses could potentially lead to a new virus with broad host specificity potentially causing a public health crisis.

8.2
Porcine Lymphotropic Herpesvirus

Porcine lymphotropic herpesvirus (PLHV) is a recently identified gamma-herpesvirus in swine. The first evidence for the existence of a new porcine lymphotropic herpesvirus was obtained by Ehlers et al. (1999). These investigators used PCR with degenerate primers representing conserved regions of the herpesvirus DNA polymerase gene to screen peripheral blood mononuclear cells (PBMC), spleens, lungs, kidneys and livers of pigs from Germany and Spain. Specific PCR fragments were amplified from several splenic and PBMC samples. Sequence analysis of PCR fragments revealed two closely related gamma-herpesviruses (PLHV-1 and PLHV-2) with a high homology with the DNA polymerase gene of herpesviruses of the oncogenic subfamily *Gammaherpesvirinae*. In a further study conducted by the same group (Ulrich et al. 1999), a 4.6-kb fragment of the genome of both PLHV-1 and PLHV-2 viruses was sequenced. The region sequenced included the 3'-end of glycoprotein B gene, the entire DNA polymerase gene and downstream ORF. Sequence comparison showed 95% amino acid identity between PLHV-1 and

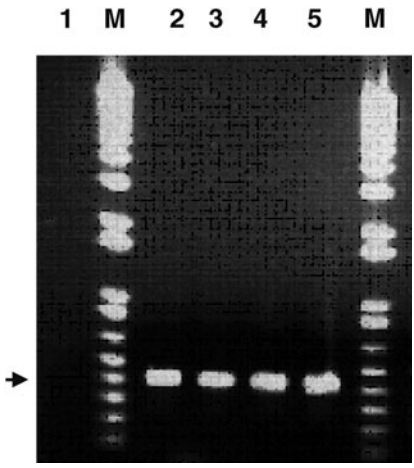

Fig. 8 Detection of PLHV by PCR in spleen of pigs. Note PLHV PCR-amplified DNA fragment of 437 bp in *lanes 2–5*. A similar band was not detected in DNA from normal spleen (*lane 1*). *Lane M* represents 1 kb molecular weight standards. All four samples were positive for PLHV by PCR

PLHV-2 DNA polymerase genes. Phylogenetic analysis showed that DNA polymerase genes of PLHV form separate clusters with other members of the *Gammaherpesvirinae* subfamily, including Epstein–Barr virus, equine herpesvirus 2, *Herpesvirus saimiri*, rhesus monkey rhadinovirus, human herpesvirus 8 associated with Kaposi's sarcoma, and murine gamma-herpesvirus 68. Another ORF located downstream of the DNA polymerase gene also was characterized. Overall, the characterized sequence showed G+C content of 37%, with suppression of CpG dinucleotide frequency. These characteristic features have been found previously in several A+T-rich lymphotropic herpesviruses of the genus Rhadinovirus (*Herpesvirus saimiri*, murine gamma-herpesvirus 68, and AlHV-1) (Albrecht et al. 1992; Ensser et al. 1997; Virgin et al. 1997).

Limited studies suggest that PLHV infections are common in swine. Ehlers et al. (1999) analyzed DNA isolated from PBMC of 39 animals and spleens of 19 animals and found 87% of PBMC and 95% of spleens were positive for PLHV. We have also evaluated 20 tissue and blood samples from eight different cases submitted to the Veterinary Diagnostic Laboratory of Iowa State University, and peripheral blood leukocytes from five animals from the Iowa State University Animal Science Swine Herd. Peripheral blood leukocytes from eight herds were positive by PCR (Fig. 7).

PLHV-1 and PLHV-2 represent two newly recognized members of the *Gammaherpesvirinae* subfamily, and represent the first gamma-herpes-

virus infecting pig species. Limited PCR studies indicate a high prevalence of these viruses in both domestic and feral pigs (Fig. 8). No information is available on modes of transmission and the ability of PLHV to cause persistent and/or latent infection. However, based on the ability of herpesviruses to cause latent infections, it is highly likely that PLHV too causes persistent/latent infections. Additional studies are needed to better characterize PLHVs, to better define their epidemiology, to determine their modes of transmission and prevalence in swine herds, and to examine the feasibility of producing pigs free of PLHV. The PLHV should be considered as a xenozoonoses risk and pigs for xenotransplantation should be screened for PLHV.

8.3 Porcine Cytomegalovirus

Porcine cytomegalovirus (PCMV) infection characterized by the presence of inclusion bodies in the mucosal glands of turbinates was described by Done (1955), who later reported herpes-like particles in lesions. The name 'inclusion body rhinitis' was given because of the presence of basophilic intranuclear inclusion bodies in the nasal passages of pigs with rhinitis. PCMV is now ubiquitous in swine populations around the world. PCMV is a beta-herpesvirus belonging to the family *Herpesviridae*. Viral particles are enveloped with a diameter of 120–150 nm.

Although PCMV infection in pigs is common, clinical disease and mortality are uncommon. The severity of disease depends on the age and immune status of the pig and immunity of the dam. The two primary modes of PCMV transmission are transplacental and by droplet. Virus invades and replicates in cells of the nasal, lacrimal, and Harderian mucous glands leading to viremia. There is a predilection for cells of the reticuloendothelial system. Virus is also shed in urine.

Clinical signs in pigs under 10 days of age include: sneezing, thick white nasal exudates, inability to nurse, paresis, and death in 4–5 days. In pigs over 2 weeks of age clinical signs include: snuffles, mucopurulent nasal exudates, low morbidity, anemia, stunting and mortality. In most pigs, infection is unapparent. Gross lesions include mucopurulent nasal exudates, sinusitis, petechial hemorrhages on the kidneys, possible mild multifocal consolidation of the lung and interlobular edema. Microscopically, typical large basophilic intranuclear inclusion bodies are seen in cytomegalic cells of the tubulo-alveolar glands of the turbinates. Occa-

sionally, similar inclusions are present in other organs, especially renal tubular epithelium, and there is desquamation of glandular epithelium, lymphoplasmacytic inflammation, loss of cilia, and lymphoid hyperplasia.

Diagnosis is based on histopathologic observation of the characteristic basophilic intranuclear inclusion bodies in the nasal mucus glands. Virus isolation is achieved by inoculation of alveolar macrophage cells from pigs with suspected material. Viral antigen can be detected by immunofluorescence using anti-PCMV serum. A porcine fallopian tube cell line has been used to adapt PCMV and used in an indirect immunofluorescence assay to detect antibodies. Recently, a PCR assay has been developed for the detection of PCMV (Fryer-Jacqueline et al. 2001). However, these techniques are not routinely available. Young pigs can be infected in the presence of maternal antibody and the infection is often subclinical. The initial site of replication is in the mucous glands of the nasal mucosa. In neonatal pigs, viremia occurs between 5 and 19 dpi, although in older pigs this period is often between 14 and 18 days. Generalized infections may occur in neonatal pigs and the virus can be found in the reticuloendothelial cells with inclusion bodies in macrophages, capillary endothelium, sinus and sinusoidal cells of lymphoid tissue (Edington 1999). In older pigs the virus is usually confined to the nasal mucosa, but has also been identified in the Harderian and lachrymal glands and renal tubules. PCMV can cross the placenta causing fetal death or generalized disease in neonatal piglets (Edington et al. 1977). The most important route of virus shedding is from the nasal secretions, but virus is also excreted in the urine and ocular (Plowright et al. 1976) and cervical fluids (Edington et al. 1977).

PCMV has been identified in pulmonary macrophages of healthy pigs, suggesting that animals may become latently infected (Edington et al. 1976a). PCMV has also been shown to be reactivated after corticosteroid treatment (Edington et al. 1976b). A PCR test based on the sequence of the DNA polymerase gene has been developed recently to detect PCMV in blood and tissue DNA samples (Hamel et al. 1999; Widen et al. 1999; Rupasinghe et al. 1999). Although a number of studies have been conducted on acute infections with PCMV, limited information is available on persistence and latent infections. Also, improved methods for the propagation and detection of this virus need to be developed.

8.3.1
Xenozoonoses

PCMV is host specific and does not replicate in cells of nonporcine origin or in rabbits, mice, hamsters, chick embryos, or cattle. The host specificity of PCMV limits its ability to be a significant xenozoonotic risk. However, human cytomegalovirus is considered an opportunistic organism and is commonly present in immunosuppressed human patients. PCMV, like other cytomegaloviruses, causes persistent infections and hence present a xenozoonoses risk. What role immunosuppression has in the susceptibility of human patients to PCMV is unknown.

9
Porcine Parvovirus

PPV is ubiquitous in the domesticated swine population worldwide. The salient features of PPV infections are that infections are unapparent except for reproductive failure in pregnant females. The virus crosses the placental barrier in pregnant females and infects fetuses in utero, resulting in embryonic death and resorption, fetal death and mummification, and reduction in the number of live born pigs per litter.

9.1
Virus Characteristics

PPV is a member of genus *Parvovirus* of the family *Parvoviridae* (Mengeling 1992). Other members of this family include human parvovirus, canine parvovirus, minute virus of mice, and feline panleukopenia virus. PPV is an enveloped virus of 20 nm diameter. The viral genome is single-stranded linear DNA of 5 Kb and replicates through a double-stranded replicative form. The viral capsid is composed of three structural proteins: VP1 of 82.5 kDa, VP2 of 65 kDa, and VP3 of 62 kDa. VP3 is the major viral immunogen and is responsible for viral neutralization. PPV possesses hemagglutination activity and guinea pig erythrocytes are the best source for erythrocytes for detecting this. The virus is very stable to heat, pH and treatment with common disinfectants. PPV requires mitotic cells for replication, a possible reason for its predilection for fetal tissues. PPV replicates in swine kidney cells and has been adapted in se-

lected established cell lines. The virus produces cytopathic effects that include cell rounding, pyknosis and cell degeneration.

The PPV genome is quite stable and well conserved, based on antigenic and genetic studies, and most of the viral isolates represent a single serotype of PPV. This is in contrast to the extensive heterogeneity among feline and canine parvoviruses. However, some antigenic and genetic variation has been observed: two PPV isolates, the KBSH and Kresse strains, have minor genetic differences from the reference strain NADL. Additionally one isolate, the H-45 strain isolated from diarrheic pigs in Japan, has been shown to be distinct.

9.2 Pathogenesis

The natural route of exposure to PPV appears to be oronasal. PPV infections are usually subclinical or result in minimal clinical disease, such as mild fever and leukopenia. PPV crosses the placental barrier and infects concepti. The outcome of PPV infection depends upon the stage of the embryo/fetus at the time of PPV infection. PPV exposure during the first 2 weeks of gestation may result in embryo death and resorption and sows may return to estrus. Infection of the conceptus during the second trimester of gestation results in fetal infection and death followed by mummification (Fig. 9). As a result, a smaller litter of pigs is born. Infection of the fetus during the third semester of gestation as the fetus becomes immunocompetent does not result in fetal death, but fetuses are infected and make antibodies. Viremia, persisting usually for 7–14 days, is detected in pigs around 3 days after infection. Virus is excreted in feces and can be detected for 28 days.

9.3 Diagnosis

PPV infection in pigs is easily detected by HI, virus neutralization, or ELISA. HI is most commonly used. Virus can be isolated in primary porcine kidney cell cultures and viral antigens can be identified by an immunofluorescence test. An immunofluorescent assay has also been often used to detect viral antigens in infected tissues or to confirm PPV diagnosis in mummified fetuses. PCR, nucleic acid probes and in situ hybridization tests are available for viral nucleic acid detection.

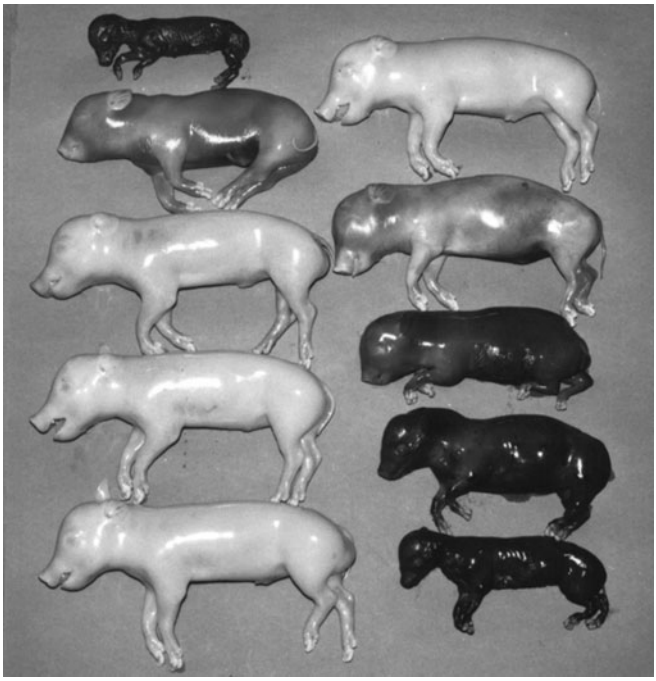

Fig. 9 Dead and mummified pigs within a litter of sows inoculated orally with PPV at 42 days of age and necropsied at 84 days of age. PPV antigen was evident in the lung sections of dead and mummified fetuses

9.4 Control and Eradication

PPV infections are widespread and almost 100% of adult pigs older than 1 year of age are infected with PPV. Pigs born to seropositive dams are protected from PPV infection and passively acquired antibodies also are protective. PPV-free pigs can be obtained from seropositive pigs by isolating them from adult pigs at around 3–4 weeks of age and raising them in clean environment free from exposure to PPV-positive pigs. Similarly, PPV-free pigs can also be obtained by Cesarean section from sows. As PPV infections are common in swine herds, extreme care should be taken to avoid PPV infection when introducing new genetic stocks.

9.5
Xenozoonoses

PPV is a common contaminant of trypsin (Croghan et al. 1973), which is derived from porcine pancreas and has been used extensively in cell culture work for production of vaccines for humans and animals. There has not been any evidence of PPV-associated disease in humans or reports of PPV-associated infections or diseases in swine handlers and veterinarians. This is in spite of the fact that porcine clotting factor has been used for many years in the treatment of hemophilia in humans. Erdman et al. (2000) found 21 of 22 lots of Hyate:C to be contaminated with PPV. None of the 98 serum samples tested from patients receiving Hyate:C treatment had antibodies to PPV. This study showed that risk of human infections with PPV is low; however, it would be wise to use PPV-free tissues for xenotransplantation as PPV produces persistent infections and crosses the transplacental barrier. The immunosuppressive properties of feline and canine parvoviruses and the adaptation of feline panleukopenia virus in dogs resulting in a major epidemic further suggest the potential of parvoviruses to cross the species barrier.

10
Coronaviruses

Four coronaviruses have been identified in pigs: transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus, porcine epidemic diarrhea virus and hemagglutinating encephalomyelitis virus. Only a brief description of these viruses, the diseases they produce, and diagnosis is provided here as they do not present a significant risk through xenotransplantation. However, there is a concern that coronaviruses present a xenozoonoses risk because of the high rate of errors during coronavirus replication through polymerase jumping, and their ability to recombine which could lead to new viruses capable of replication in both pigs and humans.

Transmissible gastroenteritis virus is the most common and best studied of the swine coronaviruses (Saif and Wesley 1999). It is a member of the family *Coronaviridae*. The virus is pleomorphic with a diameter of 160–200 nm and 12–25-nm-long surface spikes. The viral genome is about 30 kb and has three major structural proteins (S, M, and N): S is the envelope spike glycoprotein of 200 kDa, M is an integral mem-

brane protein of 28 kDa, and N is a nucleoprotein of 48 kDa. TGEV causes gastroenteritis in neonatal pigs; it replicates primarily in the respiratory and the intestinal tract, where lesions include villous atrophy. TGEV persists for a long time in pigs and it is suspected that virus-infected pigs may serve as carriers—the virus is shed in feces for about 4 weeks; however, it has been isolated from lungs for 104 days (Underdahl et al. 1975). Virus can be isolated in a swine testis cell line or can be detected by PCR, immunofluorescence, immunohistochemistry or in situ hybridization (reviewed by Sirinarumitr et al. 1997). Viral antibodies are routinely detected by a virus neutralization test.

Porcine respiratory coronavirus (PRCV) appears to be a mutant of TGEV. It was first detected by Pensaert et al. (1986) and now is believed to be widespread around the world. Major differences between PRCV and TGEV are that a portion of S gene is missing in PRCV giving rise to smaller S protein of 180 kDa. The size of the deletion in S gene varies from 621 to 681 base pairs (Paul et al. 1994). Deletions are also present in genes 3 and 3-1. PRCV also has a different tropism from that of TGEV: TGEV replicates in the villous epithelial cells of both the small intestine and the respiratory tract whereas PRCV replicates primarily in the respiratory tract in cells such as bronchiolar epithelial cells, type II pneumocytes, alveolar and septal macrophages, and type 1 pneumocytes. PRCV and TGEV can be isolated in a swine testis cell line and the two viruses can be differentiated by reverse transcriptase–PCR (Vaughn et al. 1995; Sirinarumitr et al. 1996, 1997). Antibodies to the two viruses can be differentiated by competitive ELISA (Callebaut et al. 1989).

Porcine epidemic diarrhea virus is caused by coronavirus and is antigenically related to TGEV, however, PEDV is not present in the swine herds in the USA. The disease is characterized by watery diarrhea similar to that caused by TGEV except that PEDV replicates in both small and large intestine. PEDV is fastidious and has been adapted to grow in the Vero cell line using trypsin producing extensive syncytia. There appears to be only one serotype of the virus. Pigs are the only known hosts for PEDV.

Hemagglutinating encephalomyelitis virus (HEV) is associated with respiratory and neurologic disease in pigs. HEV is similar to other coronaviruses except that it has five major proteins including hemagglutinin. The virus replicates in pig kidney cells producing syncytia. HEV also possesses hemagglutinating activity and hemagglutinates chicken and mouse erythrocytes. The virus is quite stable as there is only one known

serotype. HEV is antigenically related to Bovine coronavirus and human respiratory coronavirus OC43. HEV infections are mostly subclinical but do produce two clinical syndrome in neonatal pigs: an acute disease with encephalomyelitis or vomiting, and wasting disease. Microscopic lesions are observed in the tonsils and in the nervous and respiratory systems. Diagnosis can be made by virus isolation from tonsil, brain, and lungs. Antibodies to HEV can be detected by virus neutralization or hemagglutination inhibition tests.

Swine coronaviruses appear to be host specific. No reports are available on infections of other species with swine coronaviruses. Coronaviruses undergo mutations during replication due to polymerase jumping errors giving rise to new mutants. Porcine respiratory coronavirus is such a mutant of TGEV with different tropism. Coronaviruses have also been shown to undergo recombination. Because of their ability to recombine and mutate to give rise to new viruses with different tropisms coronaviruses should be considered a potential xenozoonoses risk.

11
Adenoviruses

Adenovirus infections are common in swine and adenoviruses appear to persist in swine tissues for long periods, but have not been shown to be associated with any significant disease. Characteristics of swine adenovirus are similar to those of other adenoviruses. Adenovirus is a member of the family *Adenoviridae*. The virus is nonenveloped, spherical and 75 nm in diameter. The viral genome is double-stranded DNA encapsulated by a protein shell comprised of 252 capsomeres. Porcine adenovirus is stable to chloroform, low pH, and temperature. Four serotypes (1–4) have been detected in pigs; serotype 4 is the most abundant. Natural infection with adenovirus is by inhalation or by the oral route. Virus transmission is via a fecal oral route and possibly by aerosol. Virus is excreted in feces and can be detected for several weeks. Experimental infection of pigs with adenovirus serotype 4 resulted in diarrhea. Lesions have also been detected in brain, lung, and kidney. Adenovirus is incriminated in respiratory disease, and is isolated from aborted fetuses. The virus replicates primarily in the tonsils and small intestine and has been shown to persist in kidneys. Meningoencephalitis was observed in pigs infected experimentally with adenovirus types 1 and 3. Heart and

liver also contained chronic inflammatory changes. The primary sites of virus replication are the tonsils.

Adenovirus should be suspected if inclusion bodies are detected in the lungs, liver, or kidneys. Demonstration of viral antigen by fluorescence assay or immunoperoxidase staining in tissues confirms the role of adenovirus in the disease. Virus isolation can be accomplished in porcine kidney cell cultures. The virus does not produce any characteristic cytopathic effect, hence the viral antigen is detected by an immunofluorescence test. Adenovirus infection can be detected by virus neutralization by rising antibody titers.

No information is available on the public health risk of swine adenoviruses. However, pigs have been shown to be susceptible to human adenovirus; infection of neonatal pigs with human adenovirus resulted in bronchopneumonia (Betts et al. 1962). No data is available on whether swine adenovirus infects humans. Adenovirus does produce persistent infection so it is prudent to screen pigs and keep them free from adenovirus infection.

12 Conclusion

There have been major advances during the last decade in our understanding of pathogenesis of swine viral infections. More reliable diagnostic tests have increasingly become available for the detection of swine viruses and swine viral infections. In spite of these advances there are major gaps in research to assess the xenozoonoses risk of swine viruses. Studies are needed to determine whether swine viruses infect humans; serologic evidence of infections with swine viruses should be sought in high-risk populations such as swine producers, veterinarians, and abattoir workers. The potential of swine viruses to infect humans can be assessed by studying their ability to replicate in human cell lines as well as primary cell cultures of human origin. Nonhuman primates may also serve as good animal models to test the ability of swine viruses to infect humans. Hence, infection of nonhuman primates with swine viruses under conditions mimicking xenotransplantation will provide additional information on their potential xenozoonoses risk. Monitoring of xenotransplant human and nonhuman primate recipients for possible infections with swine viruses is essential. Such studies will require additional

resources for the development of rapid and more reliable diagnostic methods.

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