Cultivation, preliminary characterization, and prevalence in swine herds of porcine paramyxovirus

by

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ABSTRACT

A paramyxovirus was previously isolated in primary kidney cell culture from finishing pigs with respiratory and central nervous system (CNS) disease (Janke et al., 1992). This isolate (ISU-92) was pathogenic for four 3-day-old gnotobiotic pigs; resulting in mild pneumonia and encephalitis (Janke et al., 1992). Each of six 6-week-old specific pathogen free (SPF) pigs were inoculated intranasally with 5 ml of cell-culture-propagated porcine paramyxovirus (PPMV). This inoculum had a titer of 10^7 tissue culture infectious dose $(TCID)_{50}$/ml. No gross lesions were found at necropsy. Microscopically, a severe focal vasculitis with locally extensive gliosis was noted in the brain stem of one pig. There was no evidence of interstitial pneumonia (Battrell et al., 1993).

This study was initiated to: adapt ISU-92 to a continuous cell line, determine its pathogenicity, develop a serologic test for viral antibody detection, and use this test to determine the prevalance of the virus in conventional swine herds.

Several cell lines were tested for the propagation of ISU-92. Cell line PSP-27 was determined to be susceptible to

viral infection producing multinucleated giant cells. Presence of virus was confirmed by electron microscopy.

A virus neutralization test for PPMV was developed. To test the sensitivity of the test and to determine the initial appearance and duration of antibodies detectable by this test after infection, antibody response against PPMV was measured in PPMV-inoculated SPF pigs. Anti-PPMV antibodies were first detected at 7 days post inoculation (DPI), and remained detectable in principal pigs for the duration of the experiment, or twenty-four weeks after inoculation.

The seroprevalence of PPMV in Iowa swine herds was determined using the virus neutralization test. Serum samples collected in 1988 and 1989 from thirty-six Iowa swine herds as part of a National Animal Health Monitoring Service (NAHMS) study were used. A total of 876 serum samples were tested. Six of these samples had antibodies against PPMV. Titers were low. The 6 samples were collected from five different herds.

These studies show that PPMV infection was rare in swine herds in 1988 and 1989. Analysis of recently collected serum samples will provide more accurate data on the incidence of PPMV infection in swine herds.

INTRODUCTION

Field Case

History, clinical signs and lesions

The initial outbreak of porcine paramyxovirus was described in detail by Janke et al (1992). To summarize: a large farrow-to-finish swine operation experienced an outbreak of respiratory and CNS disease in finishing hogs. The herd had been repopulated two years before using back-cross gilts. Since the repopulation, only boars were introduced to the herd. Respiratory signs began in a continuous-flow finishing barn that housed 350 pigs. All 350 pigs were reported to be affected. The disease then spread to a second finishing barn, and subsequently to the nursery, farrowing and gestation barns on an adjacent farm.

In the finishing house in which the problem first appeared, pigs ranged in weight between 150 and 190 pounds. Affected pigs had a mild cough and temperatures of 103 to 104.5° Fahrenheit. Sick pigs were treated at this time with injectable dexamethasone and tylosin. By the fourth day following the first appearance of clinical signs, a total of seventeen pigs had died. The consulting veterinarian was notified and necropsies were performed. At necropsy, pigs had

wet, heavy, congested lungs with small areas of consolidation on the ventral tips of lung lobes. No other gross lesions were noted. Affected pigs were treated with a combination of penicillin, dexamethasone, spectinomycin, and atropine. No additional pigs died following this treatment, but the infection continued to spread throughout the operation.

The disease spread to a second finishing building, where within a few days, all 400 pigs in this all-in/all-out unit were affected. Many pigs became dyspneic, a few pigs developed a harsh barking cough, while others showed signs of CNS disturbance. The consulting veterinarian reported episodes of apparent distress characterized by persistent squealing and whole-body tremors. Several pigs developed rear-end ataxia, and intermittent episodes of head-pressing. At the first onset of clinical signs, pigs in this unit were treated with injectable penicillin and dexamethasone. Morbidity was quite high, however, no death loss occurred.

The breeding, farrowing, nursery, and grower buildings were located on a second farm 1/4 mile north of the finishing barns. Pigs in the grower building were only mildly affected. Nursery age pigs were off feed for several days, and became more dyspneic than older pigs. No deaths occurred in this age group of pigs, and coughing was less severe than in the finishing building.

Nursing piglets in the farrowing barn became quite sick, but no deaths due to the disease outbreak occurred in this age group. Treatment of nursing pigs consisted of injectable antimicrobials given for three successive days followed by medicated early weaning.

In the gestation barn from a population of 141 females, a few sows and gilts went off feed during the outbreak; two or three abortions, and two premature farrowings occurred. The cause of the abortions and premature births was not investigated (Janke et al., 1992).

Diagnostic laboratory examination

Gross observations Two live 150-pound pigs from the second finishing building were submitted to the Veterinary Diagnostic Laboratory at Iowa State University. On arrival, neither pig exhibited signs of respiratory distress; however signs of a CNS disorder were pronounced. Both pigs exhibited rear-end ataxia, whole-body tremors, and a reluctance to move (Janke et al., 1992). One pig was depressed. The other pig sqealed continuously, and head-pressed the walls of the pen or the flank of the other pig. Gross lesions were restricted to the ventral portions of cranial and middle lung lobes, and

consisted of bilateral consolidation involving twenty percent of the total lung volume. No other gross lesions were found.

Microscopic lesions Histopathologic examination was conducted on samples of lung, brain, heart, liver, kidney, spleen, and intestine. Microscopic examination of the lungs revealed a moderately-severe bronchointerstial pneumonia. Numerous bronchioles had extensive necrosis and regeneration of epithelium. Mixed inflammatory cells and edema filled partially collapsed alveoli, and bronchioles and arterioles were surrounded by small cuffs of lymphocytic cells. Mild diffuse gliosis and mild vasculitis were noted around scattered vessels in all portions of the brains. No lesions were observed in other tissues (Janke et al., 1992).

Laboratory studies No significant bacteria were recovered from tissue samples collected from either pig. Frozen sections of brain, lung, spleen and tonsil were tested for pseudorabies (PRV) and swine influenza virus (SIV) using fluorescent antibody staining. In addition, virus isolation studies were conducted on pooled tissue samples by inoculation onto Mädin-Darby bovine kidney cells, swine testes cells, and embryonated chicken eggs. In both the fluorescent antibody and virus isolation test, neither PRV nor SIV was detected. Tissues were submitted to the National Veterinary Services

Laboratories (NVSL), United States Department of Agriculture (USDA) for additional virus isolation studies (Janke et al., 1992).

Virus isolation A 20% tissue suspension was created by homogenizing pooled tissues with F15 Medium. Gentamicin and amphotercin B were added to the suspension to inhibit bacterial and fungal growth. The suspension was centrifuged at 1000xg for 10 minutes and inoculated onto primary fetal porcine kidney (FPK) cells in stationary roller tubes for 1 hour at 37°C. Cell monolayers were rinsed three times with serum free medium. This was followed by incubation under F15 medium and 2.5% fetal bovine serum until cytopathic effect (CPE) was observed. Following the appearance of CPE, cells were frozen at -80°C and thawed.

A paramyxo-like virus was isolated in the cell culture on second passage and designated ISU-92. Indirect immunofluorescent assays were conducted. The isolate reacted with antiserum against parainfluenza 1, 3, and 4B viruses. ISU-92 did not produce immunofluorescence when treated with antiserum against parainfluenza 2, 4A or 5 viruses or the blue-eye paramyxovirus (Janke et al., 1992).

Pathogenicity of Porcine Paramyxovirus for Specific Pathogen Free and Gnotobiotic Pigs

Janke et al. (1992) used a lung homogenate (20% w/v tissue suspension with F15 Medium, gentamicin, and amphotericin B) from pigs in the PPMV-infected herd to inoculate two six-week-old SPF pigs intranasally. Pigs were observed daily for 10 DPI. No differences were noted between principal and control pigs in rectal temperatures, daily feed intake, or growth rates. No gross or microscopic lesions were found in either pig (Janke et al., 1992).

In further work Janke et al. (1992) inoculated four three-day-old gnotobiotic pigs intranasally with cell-culture-propagated PPMV (ISU-92). Principal pigs had slightly elevated rectal temperatures, and less vigor than control pigs. At necropsy, all 4 principal pigs had irregular areas of tan-gray discoloration of the cranioventral portions of cranial, middle, and accessory lung lobes (Janke et al., 1992). Microscopically, there was an irregular thickening of the alveolar septa due to swelling and proliferation of pneumocytes and by a light infiltration of macrophages (Janke et al., 1992). Microscopic examination of the brain revealed a few glial nodules in the midbrain of one of the principal pigs (Janke et al., 1992).

Research Questions

Viral pneumonia in swine has been reported with increased frequency. Prior to the recent discoveries of porcine reproductive and respiratory syndrome (PRRS) virus, porcine respiratory coronavirus (PRCV) and variant strains of influenza virus, only SIV and PRV had been identified with any frequency as the causative agents of swine interstitial pneumonia (Halbur et al., 1992; Paul et at., 1992). In a number of swine respiratory cases in which gross and microscopic lesions are suggestive of viral involvement, no virus has been detected. Possible explanations for the failure to detect viruses in these cases include: the lack of acutely affected pigs available for examination, masking of viral antigens by concurrent bacterial infections, or the lack of a diagnostic tests for the particular virus involved.

The isolation of PPMV from a large swine operation experiencing respiratory disease and CNS disturbances suggests this virus might be the cause of some cases of interstitial pneumonia in which no virus has been identified previously. To determine the role of PPMV in swine respiratory disease, characteristics of the virus need to be established. Clinical disease and lesions resembling those observed in the field case need to be reproduced by inoculation of healthy swine with the isolated PPMV. A practical method of diagnosis by

detecting the virus in tissues or by serological tests needs to be established. Of great interest is the prevalence of this virus in swine herds. PPMV may be responsible for many cases of viral pneumonia in swine, or infection could be a rare.

Organization of this Study

In this thesis, a review of the literature for information on the family Paramyxoviridae is presented. This is followed by pathogenicity studies on ISU-92 in SPF pigs, the development of a virus neutralization test for the detection of anti-PPMV antibodies in swine serum samples, and the determination of PPMV in Iowa swine serum samples collected in 1988 and 1989.

LITERATURE REVIEW

Introduction

It has been established that members of the family Paramyxoviridae cause of a variety of diseases affecting both humans and animals. They have been shown to be associated with disease in avian (Doyle, 1927; Bankowski et al., 1960; Tumova et al., 1979), porcine (Sasahara et al., 1954), canine (Hull et al., 1956; Binn et al., 1967; Carandell et al., 1968; Appel and Percy, 1970), human (Chanock et al., 1958; Chanock and McIntosh, 1990), bovine (Reisinger et al., 1959; Abinanti et al., 1960; Gale and King, 1961), equine (Dichtfield et al., 1965), and ovine species (Hore et al., 1968). Although recognized as important respiratory tract pathogens, some members of the family Paramyxoviridae are destructive to components of the central nervous system. Central nervous system disorders are a feature in the clinical presentation of diseases associated with measles virus and mumps virus in humans (Shaffer et al., 1942; Kilham, 1949; Scheid, 1961), Newcastle disease virus (NDV) in chickens (Brandly, 1964; McFerran and McCracken, 1988), canine distemper virus in dogs (Appel, 1987), and blue eye pig disease in swine (Stephano et al., 1981; Moreno-Lopez et al., 1986).

Members of the family Paramyxoviridae are all negative-stranded RNA viruses with a common virion structural pattern, and replication strategy (Kingsbury, 1990). They appear to have a close relationship with two other negative-strand RNA viruses: Rhabdoviruses and Orthomyxoviruses (Wiley, 1986). Paramyxoviruses and Rhabdoviruses are the only negative-stranded RNA viruses that have nonsegmented genomes (Kingsbury, 1990). The main feature distinguishing the paramyxoviruses from other virus families is the size and shape of the nucleocapsid within the virion (Kingsbury, 1990).

Classification

Paramyxoviridae have been classified into three genera: Paramyxovirus, Morbillivirus and Pneumovirus (Matthews, 1982; Kingsbury, 1990). The initial classification was based on morphological and biological criteria, which have now been supported by molecular data. The properties originally used to distinguish the three genera were antigenic cross-reactivities, and differences in two surface active particles: hemagglutinin and neuraminidase (Kingsbury, 1990). Paramyxoviruses demonstrate both neuraminidase and hemagglutination activity. Morbilliviruses exhibit hemagglutination, but lack neuraminidase. Pneumoviruses lack neuraminidase activity, are unable to agglutinate mammalian

and avian erythrocytes, and possess narrower nucleocapsids than the other two genera (Kingsbury, 1990).

The genus Paramyxovirus (PMV), for which NDV is the prototype, is divided into three main groups (Matthews, 1982; Kingsbury, 1990). The first group consists of mammalian paramyxoviruses (PMVs) including parainfluenza virus 1 (PIV1), PIV2, PIV3, PIV4A and PIV4B. The second group contains the mumps virus. Avian PMVs, the most notable being NDV, make up the third group and have been distributed into nine serotypes (Alexander, 1982; Alexander et al., 1983). Measles virus in humans, canine distemper virus, and rinderpest virus in cattle are members of the genus Morbillivirus. The genus Pneumovirus includes; respiratory syncytial virus in humans, bovine respiratory syncytial virus, mouse pneumonia virus (Kingsbury et al., 1978; Matthews, 1982), and the viral agent causing turkey rhinotracheitis (Lister and Alexander, 1986).

Virus Characteristics

Paramyxovirions follow the typical negative-stranded RNA virus structural pattern. They have a helically symmetrical ribonucleoprotein core, or nucleocapsid, which surrounds the nonsegmented single-stranded viral RNA genome. The flexible nucleocapsids are 13 to 18 nm in diameter with a central hole about 5 nm in diameter (Baumgartner et al., 1982; Sundqvist et

al., 1990; Fenner et al., 1993). This nucleocapsid is enclosed by an outer lipoprotein envelope. Paramyxovirions are pleomorphic in size and shape. Most virions are roughly spherical, measuring 150-250 nm in diameter. Larger virus particles (Choppin and Stoeckenius, 1964; Baumgartner et al., 1981; Lipkind et al., 1986; Stephano et al., 1988), and filamentous virions have also been reported (Lipkind et al., 1986). Stalk-like glycoprotein complexes or "spikes", which mediate virus attachment and penetration, can be seen projecting from the viral surface when negative staining techniques are used (Stephano et al., 1988; Kingsbury, 1990).

Complete nucleotide sequences of the Sendai virus (Blumberg et al., 1984, 1985a and 1985b; Morgan et al., 1984, and 1986), the measles virus (Alkhatib and Briedis, 1986; Bellini et al., 1985 and 1986; Richardson et al., 1986; Blumberg et al., 1988), and NDV (Chambers et al., 1986; Millar et al., 1986; McGinnes and Morrison, 1987; Schaper et al., 1988) have been determined. The genome of each paramyxovirus is of minus sense ssRNA, 15-16 kb in length. Each genome contains a set of six or more genes covalently linked in tandem (Fenner et al.,1993). Members of the genera Paramyxovirus and Morbillivirus are closely related in their genetic sequence. The largest (L) gene, a polymerase protein, is positioned near the 5'-terminus. The genes that code for

envelope glycoproteins [hemagglutinin (H), neuraminidase (N) (absent in Morbilliviruses), fusion protein (Fo), and membrane or matrix protein (M)] are clustered in the middle. The 3'-terminal region of the genome contains nucleocapsid genes designated NP, NC, or N.

Members of the genera Pneumovirus, for which portions of the genetic sequence has been determined, differ from Paramyxoviruses and Morbilliviruses by the presence of four additional genes in their genome. In addition, the position of the two major glycoprotein genes, G (attachment protein lacking hemagglutinin and neuraminidase) and Fo, are reversed, and the N gene is smaller in size (Collins et al., 1985a, b, and c).

The major functions of key viral proteins have been established. L (large) and P (phosphoprotein or polymerase) nucleocapsid proteins, together with either the NP, NC or N protein, are believed to play a role in the enzymatic processes of RNA transcription and replication (Kingsbury, 1990). Viral attachment to cells is mediated by surface glycoproteins [H, HN (hemagglutinin-neuraminidase), or G], which bind to sialic acid-containing receptors (Markwell et al., 1981 and 1985). The fusion protein (Fo), fuses the virus lipoprotein envelope with the surface membrane of the host cell, thereby allowing delivery of the viral nucleocapsid into

the host cell's cytoplasm (Scheid and Choppin, 1974). The M protein is thought to be involved in nucleocapsid-envelope recognition during virion assembly. It may also participate in the formation of the envelope (Shimizu and Ishida, 1975; Buechi and Bachi, 1982).

Antigenic Relationships

Antigenic relationships among members of the genus Paramyxovirus have been studied through the use of HI (hemagglutinin inhibition) and NI (neuraminidase inhibition) tests, using polyclonal antisera and monoclonal antibodies. Several antigenic cross-reactions between PMVs of avian and mammalian origin have been described. A low cross-reactivity by NI test has been reported for Sendai virus (murine parainfluenza virus type 1) with NDV (Brostrom et al., 1971), and for mumps and parainfluenza viruses with NDV by HI tests (Chanock and Coats, 1964; Tumova and Easterday, 1969). A paramyxovirus isolated from swine demonstrated a significant cross-reactivity with NDV by HI test (Lipkind et al., 1986).

Antigenic relationships among human paramyxovirus isolates and their cross-reactivity with non-human type PMVs have been studied. Different results are obtained when the antigenicity is based on the NP verses the HN proteins. Sendai virus, simian virus 5, simian virus 41, and bovine PI3

were demonstrated to be non-human types of PIV1, PIV2, PIV2, and PIV3, respectively (Kingsbury et al., 1978; Goswami and Russel, 1982; Ito et al., 1987; Tsurudome et al., 1989). However, with the use of monoclonal antibodies, Ray and Compans, (1986), showed extensive antigenic differences between the HN proteins of PIV3 and bovine PI3 virus. Differences between PIV2 and simian virus 5 have also been demonstrated (Randall and Young, 1988). By the use of polyclonal antisera and immunoprecipitation, Ito et al. (1987), concluded that human PIV1, Sendai virus, and human PIV3 belonged to one antigenic group, while simian virus 5, human PIV2, human PIV4, and mumps virus belonged to another antigenic group. Canine parainfluenza virus (canine PIV) was determined by HI test to cross-react with human mumps virus, but only in a one-way reaction that does not occur when canine PIV is tested with antisera against mumps virus (Rosenberg et al., 1971). In addition, canine PIV was demonstrated to cross-react with simian virus 5 virus (Rosenberg et al., 1971).

The antigenic relationships between paramyxoviruses isolated from swine and those isolated from other species have been determined. Lipkind et al. (1986) reported the isolation of a PMV strain from pigs in Israel which, although a different virus, was antigenically very closely related to

avian PMV serotype 3 according to HI and NI test. However, the porcine paramyxovirus which is currently endemic in central Mexico [La Piedad, Michoacan virus (LPMV)] demonstrates no cross-reactivity with antisera to bovine PI3 virus, NDV, respiratory syncytial virus, mumps and measles viruses, and human PIV1, PIV2 and PIV3 (Moreno-Lopez et al., 1986). The structural proteins of this virus were determined by the use of SDS-PAGE, and compared to those of bovine PI3 virus, NDV and Sendai virus (Sundquist et al., 1990). The mobility of the P protein of LPMV was strikingly similar to that of NDV. LPMV was similar to bovine PI3 in the mobility of the HN and NP proteins. However, the differences in genetic sequence led researchers to suggest that LPMV is a genuine porcine virus and should be classified as a novel member of the genus Paramyxovirus.

Transmission

The main route of paramyxovirus transmission is through close aerosol contact between susceptible populations and subclinically infected animals or people. This is true for bovine PI3 virus (Woods, 1968), human parainfluenza viruses (Chanock and McIntosh, 1990), NDV (Cross, 1991), and blue eye disease in swine (Stephano, 1992). Mechanical transfer of NDV is facilitated by rodents and prevailing winds. The carcasses

of dead birds infected with NDV are another source of virus (Cross, 1991). The virus responsible for blue eye disease in swine may also be disseminated by contaminated people and vehicles. Although not substantiated, winds and birds are another suspected source of transmission of this virus (Stephano, 1990).

Of great interest is the question of interspecies transfer of viruses belonging to Paramyxoviridae to an aberrant host. Humans are susceptible to infection with NDV which usually results in the development of a mild conjunctivitis. Agents resembling canine distemper virus (CDV) have been isolated from dolphins in the Mediterranean Sea (Domingo et al., 1990), and from harbor seals in the North Sea (Osterhaus et al., 1988) and the Baikal Lake (Osterhaus et al., 1989). Monoclonal antibody studies demonstrated that, although they differed in some epitopes, a close antigenic relationship exists between the virus isolated from seals in the North Sea and CDV (Orvell et at., 1990). The virus isolated from seals in the Baikal Lake was almost identical to CDV (Visser et al., 1990). A virus that differed from CDV only by reaction with one CDV monoclonal antibody was isolated from the brains of three javelinas (wild boars) with clinical signs of encephalitis in southwest Arizona (Appel et al., 1991). Although clinical disease in CDV-infected javelinas is

uncommon, in one study, 20 of 33 clinically normal javelinas had neutralizing antibodies against CDV (Appel et al., 1991). Javelinas (Tayassuidae) showed clinical signs when experimentally inoculated with another Morbillivirus, the rinderpest virus (RPV), however clinical signs and virus shedding were not observed in RPV-infected-domestic boar (Suidae) (Dardiri et al., 1969).

Clinical Signs

The clinical signs associated with infection with members of the genus Paramyxovirus are similar across species. Paramyxoviruses have a tropism for cells of the respiratory and central nervous systems.

Group one, the mammalian PMVs (PIV1, 2, 3, 4A and 4B) occur worldwide in a variety of species. Parainfluenza viruses (types 1, 2, and 3) are a major cause of croup (severe acute laryngotracheobronchitis), and pneumonia and bronchiolitis (type 3) in infants and children (Chanock, 1956; Chanock et al., 1958; Kim et al., 1961; Glezen and Denny, 1973; Welliver et al., 1986). Type 4 parainfluenza virus has been shown to cause only mild upper respiratory tract illness in young adults (Chanock and McIntosh, 1990). In addition to humans, type 1 parainfluenza viruses produce natural infections in monkeys, guinea pigs, rabbits, rats and mice

(Fenner et al., 1993). Infections usually remain subclinical, however severe respiratory disease has been reported in PIV1-infected laboratory rats and mice (Fenner et al., 1993).

Canine parainfluenza virus 2 has been isolated from dogs with respiratory disease (Binn et al., 1967; Carandell et al., 1968; Appel and Percy, 1970), and is one of several etiologic agents of kennel-cough. Clinically affected dogs exhibit signs ranging from a dry cough (Appel and Percy, 1970), to pneumonia characterized by signs of fever, malaise, and coughing with copious amounts of nasal discharge (Rosenberg et al., 1971). A canine PIV was isolated from the cerebrospinal fluid of a dog with a history of incoordination and posterior paresis by Evermann et al., (1980). This isolate induced internal hydrocephalus with signs of CNS depression and a severe inspiratory-expiratory dyspnea in dogs following experimental inoculation (Baumgartner et al., 1982).

A hemagglutinating virus was isolated from nasal mucus of calves showing clinical signs of shipping fever including: rapid respiration, nasal discharge, cough, lacrimation, conjunctivitis, inappetence and elevated temperatures (Reisinger et al., 1959). This isolate was later designated bovine parainfluenza virus type 3, and determined to be a member of the third serotype of mammalian parainfluenza viruses (Andrewes et al., 1959; Goswami and Russell, 1982).

According to Fenner et al. (1993), "antibodies to PIV3 have been demonstrated in humans, cattle, sheep, water buffaloes, deer, pigs, dogs, cats, monkeys, guinea pigs, and rats". The virus has been recovered from clinically normal cattle (Betts et al., 1964), however, evidence of its pathogenic potential does exist. Susceptible calves experimentally exposed to aerosolized bovine PI3 produced clinical signs similar to those reported by Reisinger (Reisinger et al., 1959; Gale and King, 1961; Dawson et al., 1964). It is generally accepted that bovine PI3 virus expresses its pathogenic potential in concert with other viral agents, as it is commonly isolated along with other viruses and Pasturella species from feedlot cattle with clinical signs of shipping fever (Woods, 1968). A wide range of factors including: environmental temperature, transportation, hygiene, stocking density, co-mingling, host immune status, etc., contribute to increased susceptibility to secondary bacterial invaders, and the severity of clinical disease.

A wide spectrum of clinical presentations are seen with mumps virus infections in humans. Approximately 30% of all mumps are subclinical (Center for Disease Control Mumps - United States, 1984-1986). When present, the first symptoms are usually related to infection of the parotid gland which is reported to occur in 95% of all symptomatic cases (Philip et

al., 1959). The CNS is a common target for more virulent strains of mumps, where viral invasion is presumed to occur across the choroid plexus (Wolinsky et al., 1976). Mumps meningitis is characterized by fever, headache, neck stiffness, vomiting, and lethargy (Scheid, 1961; Azimi, 1969). Seizures occur in 20-30% of patients with CNS symptoms (Azimi, 1969), however, mumps associated meningoencephalitis is rarely fatal. The virus also disseminates to the kidneys. Abnormalities in renal function are usually of minimal significance, however viruria can be detected in most patients for as long as 14 days after the onset of clinical symptoms (Utz et al., 1958). Orchitis occurs in at least 25% of mumps infected males with an increased incidence after puberty (Philip et al., 1959). Other clinical symptoms which may occur with mumps virus infections include: pancreatitis (Feldstein et al., 1974), arthritis (Gordon and Lauter, 1984), abortion (Philip et al., 1959), renal dysfunction (Utz et al., 1964), deafness (Kirk, 1987), obstructive hydrocephalus (Bistrian et al., 1972), and transient electrocardiogram abnormalities (Arita et al., 1981).

As previously mentioned, avian PMVs, the most notable being NDV, constitute the third group of paramyxoviruses. NDV (PMV-1) is probably one of the most important avian pathogens known. Clinical signs of Newcastle disease vary depending on

the tissue tropism, and virulence of the virus strain, and the level of susceptibility of the avian order infected (Cross, 1991). Most devastating are diseases resulting from viscerotropic velogenic NDV, in which 100% morbidity and mortality are not uncommon. The disease progresses rapidly, with infected birds becoming anorexic and listless, and developing cyanotic and edematous combs and wattles. Signs of severe respiratory distress and conjunctivitis soon follow (Cross, 1991). Nervous signs may be seen in older birds in advanced stages of the disease (McFerran and McCracken, 1988). Disease caused by the less virulent mesogenic NDV result in respiratory signs, CNS signs, and a decrease in egg production. Clinical manifestations of lentogenic NDV infections are typically milder than those of mesogenic NDV infections, and signs of CNS disorders are rarely present (Beard and Hanson, 1984). Turkey flocks infected with PMV-3 are reported to exhibit signs of respiratory distress and a decrease in egg production. Results of serologic studies conducted in California breeder and meat turkey flocks indicate that the prevalence of PMV-3 infected turkey flocks exceeds the incidence of clinical disease, and many infected birds remain clinically silent (Ianconescu et al., 1984).

Paramyxoviruses have been isolated from pigs with encephalomyelitis in Japan (Sasahara et al., 1954) and Canada

(Greig et al., 1971). The paramyxovirus isolated by Lipkind et al. (1986) was recovered from nasal swabs during a surveillance of Israel's swine population. The most thoroughly studied paramyxovirus in swine is the agent responsible for blue eye pig disease in Mexico. An excellent review of this disease of swine is provided by Stephano (1990). Clinical signs associated with this paramyxovirus infection are characterized by CNS disorders, reproductive failure, and corneal opacity. Nursing piglets less than 15 days of age are most susceptible. Affected pigs develop a fever and show progressive nervous signs including: weakness, ataxia, and muscle tremors (Stephano, 1990). CNS disturbances may be exaggerated when pigs are handled. Conjunctivitis, swollen eyelids, corneal opacities and lacrimation are reported in some pigs of this age group. In Stephano's review of field cases, 20 to 65 percent of litters farrowed during an outbreak of blue eye disease were affected. Morbidity ranged between 20 and 50 percent, and mortality of those affected between 87 and 90 percent. Most sows remain clinically normal although moderate anorexia and corneal opacity have been reported. Increases in the number of pregnant sows returning to estrus, and in the number of stillborn and mummified fetuses, are reported. In boars, there is a reduction in

fertility associated with orchitis and epididymitis, followed by testicular atrophy.

Lesions

Gross and microscopic lesions resulting from infection with viruses belonging to the genera Paramyxovirus are similar across species. In addition to acute laryngotracheobronchitis, infection with human PIV1 and PIV2 may extend to the lower trachea and bronchi where accumulation of inspissated mucus result in atelectasis of the lung parenchyma and pneumonia (Chanock and McIntosh, 1990). When severe disease is produced by human PIV3, bronchopneumonia, bronchiolitis, or bronchitis are reported histopathologic lesions (Parrott et al., 1962). Interstitial pneumonia, atelectasis, bronchopneumonia, bronchiolitis, and bronchitis have also been reported to occur in dogs infected with canine PIV (Rosenberg et al., 1971). Other lesions in canine PIV-infected dogs include; catarrhal rhinitis (Rosenberg et al., 1971), acute encephalomalacia, nonsuppurative meningoencephalitis and internal hydrocephalus (Baumgartner et al., 1982).

Gross lesions reported in cattle experimentally infected with bovine PI3 include: consolidation of the anteroventral portions of the lungs (Betts et al., 1964; Marshall and Frank,

1975), congestion of respiratory mucosa, and enlargement of the bronchial and retropharyngeal lymph nodes (Marshall and Frank, 1975). Histopathologic findings in affected lung lobes include an infiltration of the alveoli and bronchioles with mononuclear and polymorphonuclear cells, and multinucleated giant cell formation (Tsai and Thomson, 1975).

Gross lesions associated with velogenic and mesogenic pathotypes of NDV include: airsacculitis, lung edema, and the presence of serous to catarrhal or hemorrhagic exudate in the trachea (Cross, 1991). The ovary contains congested, degenerating follicles (Cross, 1991). Lesions resulting from viscerotropic velogenic NDV infections differ from other paramyxovirus infections in that necrotic or hemorrhagic lesions are seen throughout the alimentary tract (Cross, 1991). Necrotic foci may also be present in lymphatic tissue, and the intestinal mucosa. On histologic examination, hyperemic changes are reported to occur in most organs (Fenner et al., 1993).

No specific gross changes are consistently produced by blue eye pig disease (Stephano et al., 1988). As the name implies, corneal opacities have been observed. Other ocular lesions include: conjunctivitis, chemosis, and corneal ulcers (Stephano, 1990). A mild pneumonia is often observed in the ventral aspects of the anterior lung lobes. Histopathologic

examination of affected areas reveals scattered foci of thickened alveolar septa by the infiltration of mononuclear cells (Stephano et al., 1988). However, multinucleated giant cell formations, like those observed in the lungs of bovine PI3-infected cattle, have not been reported. Young piglets infected with blue eye pig disease may have mild gastric distention, urinary bladder distention, and small accumulations of fluid with fibrin in the peritoneal cavity (Stephano, 1990). Congestion of the brain is a common gross lesion. Microscopic examination reveals a nonsuppurative encephalomyelitis, with diffuse gliosis, neuronal necrosis, neuronophagia, perivascular cuffing, and choroiditis (Ramirez and Stephano, 1982). Grossly, affected boars may exhibit orchitis and epididymitis; which often progresses to testicular atrophy (Stephano, 1990).

MATERIALS AND METHODS

Transmission Electron Microscopic Examination of Porcine Paramyxovirus

A monolayer of fetal porcine kidney (FPK) cells was prepared in a 75-cm^2 screw-capped tissue-culture flask and washed three times with 4 ml of phosphate buffered saline (PBS). One-half ml of the second passage of isolate ISU-92 was mixed with 10 ml of serum free Delbecco's Modified Eagle Medium (DMEM) (Gibco Laboratories) and added to the FPK cell monolayer. The flask was incubated for 60 minutes at 37°C and rocked every 15 minutes to distribute the virus across the cell monolayer. Seventy ml of DMEM, with .7 ml of Antibiotic-Antimycotic [contains: 10,000 units/ml of penicillin G sodium, 10,000 ug/ml streptomycin sulfate, and 25 ug/ml amphotericin B as Fungizone] (Gibco Laboratories), and 2% bovine fetal serum were added to the flask. The ISU-92 infected cells were incubated for 48 hours. The flasks were stored frozen at -80°C.

Five ml of culture fluids were centrifuged at 2000xg for 20 minutes. The supernatant was removed by aspiration, added to 2 ml of distilled water and centrifuged at 25,000xg for an additional 4 hrs. The supernatant was discarded. One ml of

the remaining pellet was added to 40 microliters of serum collected from pig number 357 in the gnotobiotic pig trial. This serum was later determined to have a virus neutralization titer of 1:256 for PPMV.

The virus suspension and antiserum were stored at 4°C for 12 hrs, and recentrifuged at 2000xg for 20 minutes. The suspension was added at a ratio of 1:10 to a mixture containing 1 ml of 1% bovine serum albumin and 15 ml of phosphotungstic acid solution. A nebulizer was then used to spray this solution onto carbon coated grids for viewing by a Hitachi H 500 electron microscope (Hitachi Instruments Incorporated).

Adaptation of Porcine Paramyxovirus for Growth in a Continuous Cell Line

A total of five cell lines were evaluated to support the growth of PPMV: swine testicle cells (ST cells), porcine kidney cells (PK-15 cells), Mengeling-Vaughn Porcine Kidney cells (MVPK cells), Madin-Darby canine kidney cells (MDCK cells), and PSP-27 cells. Each cell line was grown to confluence in 25-cm^2 screw-capped tissue culture flasks with a growth medium of 90% Eagle's minimal essential medium (MEM)(Gibco Laboratories) and 10% heat-inactivated fetal

bovine serum. Three ml of 0.1% DEAE-Dextran (Sigma Diagnostics) were added to each flask, and incubated for 30 minutes prior to virus infection. This pretreatment of cells was used to enhance virus infectivity. A control flask of each cell line was treated with DEAE-dextran for 30 minutes. The DEAE-dextran was removed by aspiration and cell monolayers were washed three times with 5 ml of serum free MEM. One ml of stock virus, previously grown on FPK cells, was added to one flask of each cell line at a dilution of 1:100 in serum free MEM with antibiotic-antimycotic. This stock virus had a titer of 1×10^8 $TCID_{50}$/ml. The flask was incubated for 60 minutes at 37°C. Virus infected and control cultures were fed with 5 ml MEM containing 2% serum and 2.5 units of trypsin, incubated at 37°C, and observed daily for CPE. At 72 hours post infection (HPI), all flasks were frozen at -80°C.

Each infected flask was frozen and thawed three consecutive times. Subsequent passages were made for each cell line using one ml of culture fluids harvested from the previous passage. Trypsin was not used beyond the second passage as it destroyed both virus infected and control cell monolayers 24 hours following its addition.

Plaque Purification of Porcine Paramyxovirus

Monolayers of PSP-27 cells were prepared in four 6-well plates and treated with 0.1% DEAE-dextran for 30 minutes. The DEAE-dextran was removed by aspiration and the cells were washed three times with 2 ml of serum free MEM. A stock of virus was made by decanting 5 ml of the seventh passage of PPMV grown on PSP-27 cells into a sterile snap cap tube. The tube was centrifuged at 2000xg for 20 minutes and the supernant was serially diluted up to 10^{-11}. Two hundred microliters of each virus dilution was added to two wells of the 6-well plates and incubated for 60 minutes at 37°C. Two wells served as uninfected controls. The plates were rocked every 15 minutes to distribute the virus across the cell monolayer. Sea Plaque agar (FMC BioProducts), 2X-basal media (2X-BME) and bicarbonate were placed in a water bath and heated to 56°C. Eight hundred microliters of bicarbonate were added to 60 ml of a 50:50 mixture of Sea Plaque agar and 2X-BME. The virus was removed by aspiration, and 2 ml of the above mixture (agarose) were added to each well. The plates covered with agarose were left at room temperature for 20 minutes until the agarose had hardened. They were then incubated at 37°C for five days, and observed daily for CPE.

At 24 HPI, CPE was noted in wells containing the first six dilutions. On the fifth day following infection, agarose

over a plaque was cut and the virus in a well containing a 10^{-5} dilution was pipetted off, and added to a snap cap tube containing 1 ml of serum free MEM. The snap cap tube was stored at -80°C, and its contents were frozen and thawed three consecutive times. A cell monolayer was prepared in a 25-cm^2 screw-capped tissue-culture flask, and treated with 0.1% DEAE-dextran for 30 minutes. One ml of the virus in plaques mixed with MEM was added to the flask and the flask was incubated for 72 hours. The virus infected cells were frozen and thawed three consecutive times before being used as the virus inoculum for the next passage. This procedure was repeated for three consecutive passages.

Following the third passage of the plaque purified virus, 1 ml each of harvested culture fluids were added to monolayers of PSP-27 cells in four 72-cm^2 screw-capped tissue-culture flasks, and incubated at 37°C for 60 minutes. MEM containing 2% serum was added and the flasks were incubated for an additional 72 hours. These flasks were frozen at -80°C. The contents of one flask were allocated to 1 ml snap-cap tubes, and served as the virus stock for all subsequent testing.

Virus Titer Determination of Plaque Purified Porcine Paramyxovirus

The plaque purified stock virus was titrated in a series of ten fold dilutions using serum free MEM. One hundred microliters of each virus dilution was added to monolayers of PSP-27 cells in four rows of a 96-well plate. The monolayer of PSP-27 cells had previously been treated with 0.1% DEAE-dextran for 30 minutes, and rinsed three times with serum free MEM. Four rows of the 96-well plate served as uninfected controls. The plate was incubated at 37°C for 72 hours, and observed daily for CPE. At the end of 72 hours, the fluids were removed from each well by aspiration, and the cells were fixed by adding 100 microliters of methanol for 10 minutes. Cells were stained using MayGrünwald Stain (Sigma Diagnostics) to enhance visualization of virus-induced CPE.

Porcine Paramyxovirus Growth Curve

The growth curve for porcine paramyxovirus grown in PSP-27 cells was established. Cell monolayers were prepared in thirty-eight 25 cm^2 screw-capped tissue-culture flasks. Each flask was treated with 0.1% DEAE-dextran for 30 minutes, and rinsed with serum free MEM. One ml of a 1:10 dilution of plaque purified PPMV stock in serum free MEM was added to 36

of the 38 flasks. The two remaining flasks served as uninfected controls. All flasks were incubated at 37°C for 60 minutes. The cultures were fed with 4 ml of MEM containing 2% serum, and incubated at 37°C. Two flasks were stored at -80°C at each 6 hour interval up to 60 HPI. Subsequent flasks were frozen at 12 hour intervals from 60 to 120 HPI.

Each flask was frozen and thawed three consecutive times prior to the determination of the virus titer. The culture fluids harvested from each flask were diluted with serum free MEM in a series of ten fold dilutions up to and including 10^{-8}. Each dilution was added to three separate wells of a 96-well plate containing cell monolayers of PSP-27 cells. The virus titer for each flask was determined in triplicate. The 96-well plates were incubated at 37°C for 48 hours. The medium in each well was removed by aspiration, and the cells were fixed by adding 100 microliters of methanol for 10 minutes. The methanol was discarded and the cells were stained with May-Grünwald Stain to enhance visualization of virus induced CPE.

Development of Virus Neutralization Test

The presence of a characteristic PPMV-induced CPE in monolayers of PSP-27 cells provided a method for the detection of neutralizing antibodies against PPMV in swine serum

samples. Although both fluorescent antibody test and immunoperoxidase techniques were evaluated, the virus neutralization method proved to be the most dependable. The specificity of the test was evaluted using swine serum samples that were determined by the Iowa State University Diagnostic Laboratory to have neutralizing antibodies against SIV, PRRSV, TGEV and PRV. A protocol for virus neutralization was standardized, and used to test swine serum samples collected from research trial pigs, submitted field cases, and farms participating in the NAHMS study.

All serum samples to be tested were first heated to 56°C for 30 minutes to inactivate any complement components. The serum sample was diluted to the desired concentration using serum free MEM. Plaque purified PPMV stock virus with a titer of $10^{6.33}$ $TCID_{50}$/ml was added to the diluted serum samples to obtain 500 $TCID_{50}$ per sample, and incubated at 37°C for 60 minutes. This allowed PPMV specific antibodies to bind to and neutralize the virus. The virus:serum mixture was added to a previously prepared monolayer of PSP-27 cells. Infected cell monolayers were incubated for 2 hours before the virus:serum solution was discarded. MEM containing 2% serum was added to support cell growth during their subsequent incubation period of 48 hours. CPE was typically present by 24 HPI if the virus

had not been neutralized by serum antibodies, however, the CPE was more easily identified at 48 HPI. At 48 HPI, cells were fixed by adding 100 microliters of methanol at room temperature for ten minutes. Cells were then stained using May-Grünwald Stain, and evaluated microscopically for the presence or absence of CPE.

Pathogenicity of Porcine Paramyxovirus for Specific Pathogen Free Pigs

Nine 6-week-old pigs were purchased from a certified SPF herd and housed in one of two isolation units at ISU Veterinary Medical Research Institute. These pigs were free from lice, mange, swine dysentery, turbinate atrophy and snout distortion, pnuemonic lesions at slaughter; including Mycoplasma pneumoniae and Hemophilus pneumonia, PRV, Brucellosis, transmissible gastroenteritis, and PRCV. On the day following arrival, six of the pigs were inoculated intranasally with 5 ml each of FPK cell-culture-propagated PPMV. This inoculum had a titer of 1×10^{7} $TCID_{50}$/ml. Three control pigs were inoculated intranasally with 5 ml each of uninfected cell culture media. All pigs were observed daily and rectal temperatures were taken daily for 10 DPI. One principal and one control pig were euthanized at 3 DPI. One

principal pig died at 5 DPI. One principal pig and two control pigs were euthanized at 10 DPI. The remaining three principal pigs were euthanized at 24 weeks PI. Necropsies were performed and samples of brain, nasal turbinate, tonsil, lung, liver, kidney, adrenal gland, tracheobronchial lymph node, and spleen were collected for histopathologic examination and virus isolation. Blood samples were collected from principal and control pigs on 0, 3, 5, 7, and 10 DPI. Blood samples were collected from the remaining three principal pigs on 14, 21, 28 DPI and alternating weeks up to 24 weeks PI. All blood samples were maintained at room temperature for 2 hours, refrigerated for approximately 10 hours, and centrifuged at 2000xg for 15 minutes. This was followed by separation of blood from the serum clot. Serum samples were stored in 6 ml snap cap tubes at -20°C until further use. Serum antibody titers against PPMV were determined using the virus neutralization test.

Detection of Antibodies in Sera of Pigs Inoculated with Porcine Paramyxovirus

Serum samples collected from principal and control pigs in the SPF pig trial were tested for the presence of antibodies against PPMV using the virus neutralization test

described in the previous paragraph. All serum samples were diluted in a series of two fold dilutions ranging from 1:2 to 1:4096. The plaque purified PPMV stock used was diluted to 500 $TCID_{50}$ per sample or final dilution.

Determination of Seroprevalence in Iowa's Swine Population

A total of 876 serum samples were collected from Iowa swine herds in 1988 and 1989 (NAHMS Iowa pilot project, Round III). Samples were collected from fifteen to twenty-five pigs at each of the thirty-six farms involved in this survey. Serum samples were allocated and stored at -80°C for further use.

NAHMS samples were thawed and placed in a 56°C water bath for 15 to 30 minutes to inactivate any complement components. Samples were diluted with serum free MEM. Initially, serum samples were diluted and tested at a 1:8 dilution. This decision was based on the results of antibody titers against PPMV in the serum samples of SPF pigs. Plaque purified PPMV stock solution was diluted to 1:1000 and added to each serum dilution. The virus:serum mixture was incubated for 60 minutes at 37°C.

Twenty four hours prior to infection, PSP-27 cells were added to 96-well plates. The cell monolayers were washed with serum free MEM. The serum free MEM was removed by aspiration

prior to the addition of 100 microliters of serum:virus mixture. Each serum sample was added to two wells of the 96-well plate. Serum collected from pig number 357 in the gnotobiotic pig trial at 77 days after infection was diluted, and used as a positive control. A minimum of 24 wells served as uninfected controls. Once the serum:virus mixture was added to the 96-well plates, the plates were incubated at 37°C for 48 hours. They were fixed by adding 100 microliters of methanol to each well at room temperature for 10 minutes. Cell monolayers were stained with May-Grünwald Stain to enhance visualization of virus-induced CPE.

RESULTS

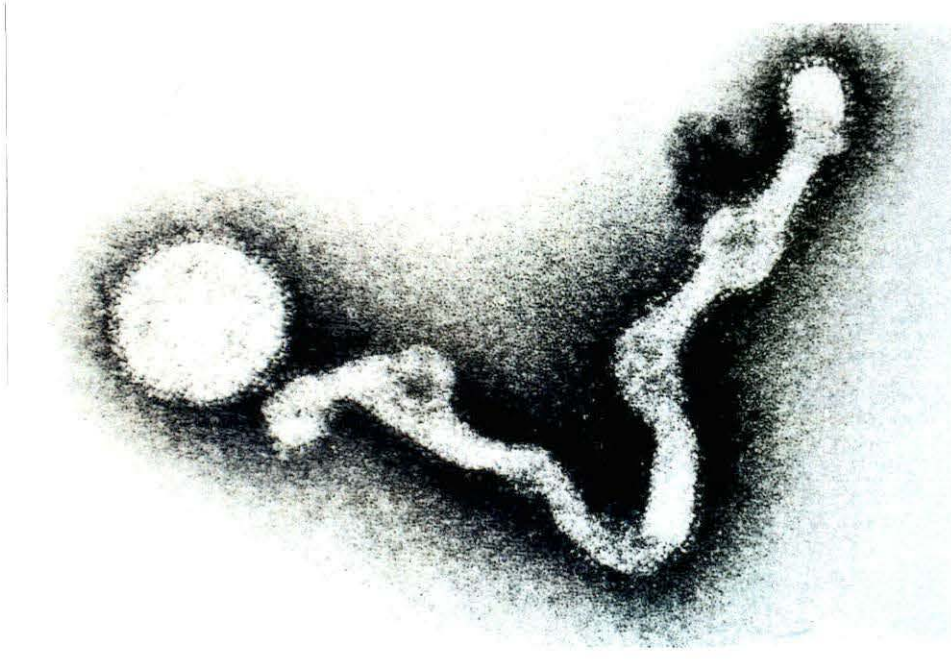
Transmission Electron Microscopic Examination of Porcine Paramyxovirus

A transmission electron micrograph of a virion found in FPK cells inoculated with ISU-92 is shown in Figure 1. This preparation was negatively stained with phosphotungstic acid, and showed an enveloped virion studded with fine surface projections, or viral spikes. The diameter of the spherical particle was approximately 250 nm. Other PPMV particles observed were pleomorphic in size and shape, with diameters ranging from 150 to 250 nm. This morphology was consistent with descriptions of other members of the family Paramyxoviridae (Kingsbury, 1990).

Propagation of Porcine Paramyxovirus in a Continuous Cell Line

ST cells were infected with the paramyxovirus isolate ISU-92 and continued for a total of eighteen passages. No CPE was noted in virus-infected cells.

The PK-15, MVPK and MDCK cell lines were inoculated with PPMV grown on FPK cells, and passed three times. No visible CPE was produced in any of these cell lines. The disrupted cells and media suspension at third passage on each cell line



Fiqure 1. Immuno-electron micrograph of porcine paramyxovirus. The diameter of the sperical partical is approximately 250 nm.

were diluted in a series of 10 fold dilutions up to 10^{-8} and used to infect FPK cell monolayers in 8 well plates. Fluorescent antibody (FA) tests were conducted on FPK cell monolayers inoculated with each of the dilutions using serum collected from gnotobiotic pig number 357 as the primary antibody. No difference was noted between control wells and those which contained dilutions of the third passage of PPMV-infected cell lines. It was therefore concluded that these cell lines did not support the growth of PPMV.

PSP-27 cells were infected with PPMV and passed three times. On the third passage a well defined CPE with syncytium formation was observed. Multinucleated syncytial giant cell formation became apparent at 24 HPI. These syncytia appeared to be cells in which the cytoplasm had failed to divide leaving a single enlarged cell with 4 to 5 nuclei. At 48 HPI, these cells had increased in diameter and had approximately 10 to 25 nuclei per cell (Figures 2 & 3). Present also, were smaller multinucleated syncytial giant cells similar to those described at 24 HPI. Cell lysis began at 72 HPI, and involved the rounding and vacuolation of virus-infected giant cells, followed by detachment from the culture vessel. By 96 HPI, approximately 50% of the cells were detached from the monolayer. At this time, the flask was

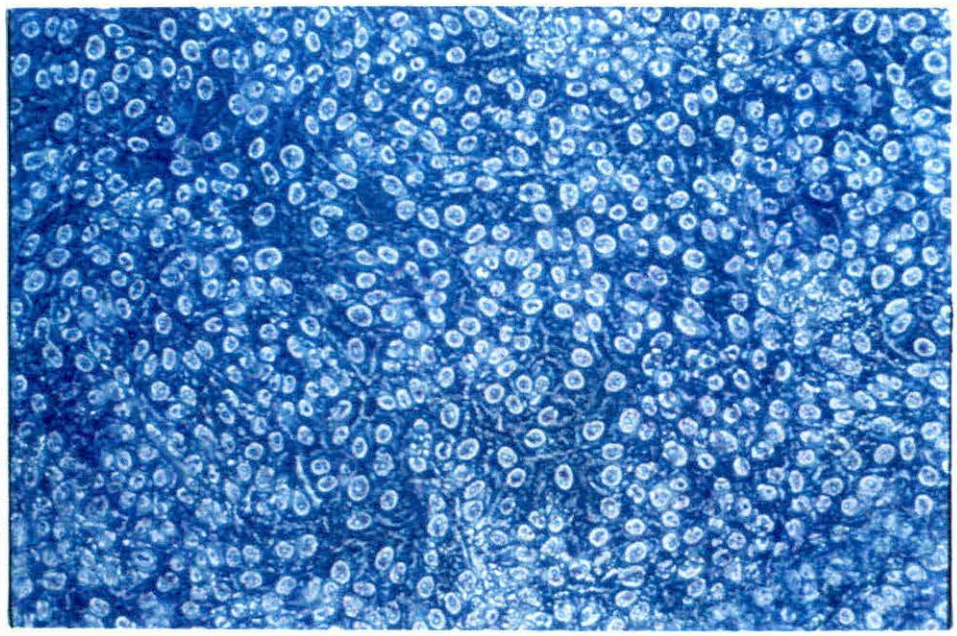
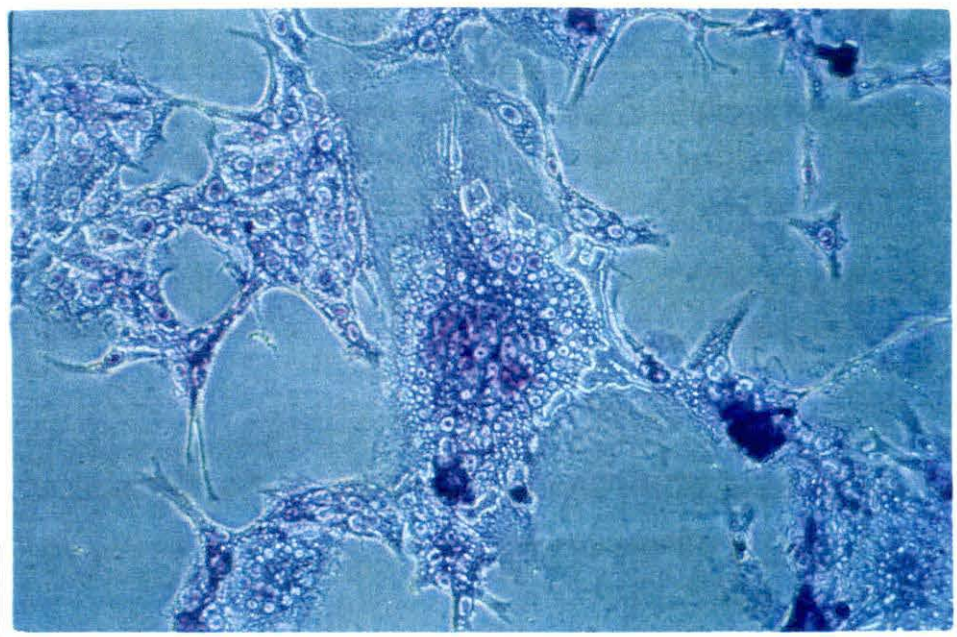


Figure 2. Uninfected monolayer of PSP-27 cells stained with May-Grünwald Stain. Magnification of 20X.



Fiqure 3. Multinucleated giant cells induced in a PSP-27 cell monolayer 48 hour following infection with porcine paramyxovirus. Stained with May-Grünwald Stain. Magnification of 20X.

frozen and used for additional passages of virus in this cell line.

The obvious CPE noted in the PPMV-infected PSP-27 cell line on the third passage resulted in the termination of subsequent passages in other cell lines. Efforts at this time were devoted toward enhancing PPMV growth in the PSP-27 cell line. With additional passages, virus-induced CPE occurred at 12 HPI. The virus was passed seven times in PSP-27 cells before being plaque-purified.

Virus Titer Determination of Plaque Purified Porcine Paramyxovirus

Syncytial cells were not present in the uninfected control wells. Characteristic PPMV-induced CPE was noted in all four replications of cells infected with dilutions 10^{-1} to 10^{-5}. CPE was not present in cells infected with dilutions of 10^{-6} or greater.

The plaque purified PPMV was determined to have a $TCID_{50}$/ml of $10^{6.33}$ using the formula devised by Reed and Muench (1938) to determine the 50% endpoint:

$$\text{Proportionate distance} = \frac{\text{distance a}}{\text{distance b}}$$

$$= \frac{\text{\% infected at dilution next ABOVE 50\% - 50\%}}{\text{\% infected at dilution next ABOVE 50\%} - \text{\% infected at dilution next BELOW 50\%}}$$

Therefore;

$$PD = \frac{75-50}{75-0} = \frac{20}{75} = 0.33 + 5 = 10^{5.33}\ TCID_{50}/100\ \text{microliter}$$

or $10^{6.33}\ TCID_{50}/ml$

Porcine Paramyxovirus Growth Curve

The growth curve for PPMV in PSP-27 cells is presented in Figure 4. PPMV was detectable 6 hours following inoculation onto a monolayer of PSP-27 cells. Virus titers increased linearly, peaking at 42 HPI ($10^{6.5}$). A virus titer of 10^4 $TCID_{50}$/ml was present in the last flasks frozen at 120 HPI. Using the general linear models procedure in the Statistical Analysis System software, the standard error between replications for a given HPI ranged between 0.167 and 0.563.

Virus Neutralization Test

The virus neutralization test was determined to be specific for the detection of antibodies in swine serum samples against PPMV. Serum samples which were determined to have neutralizing antibodies against SIV, PRRSV, TGEV and PRV

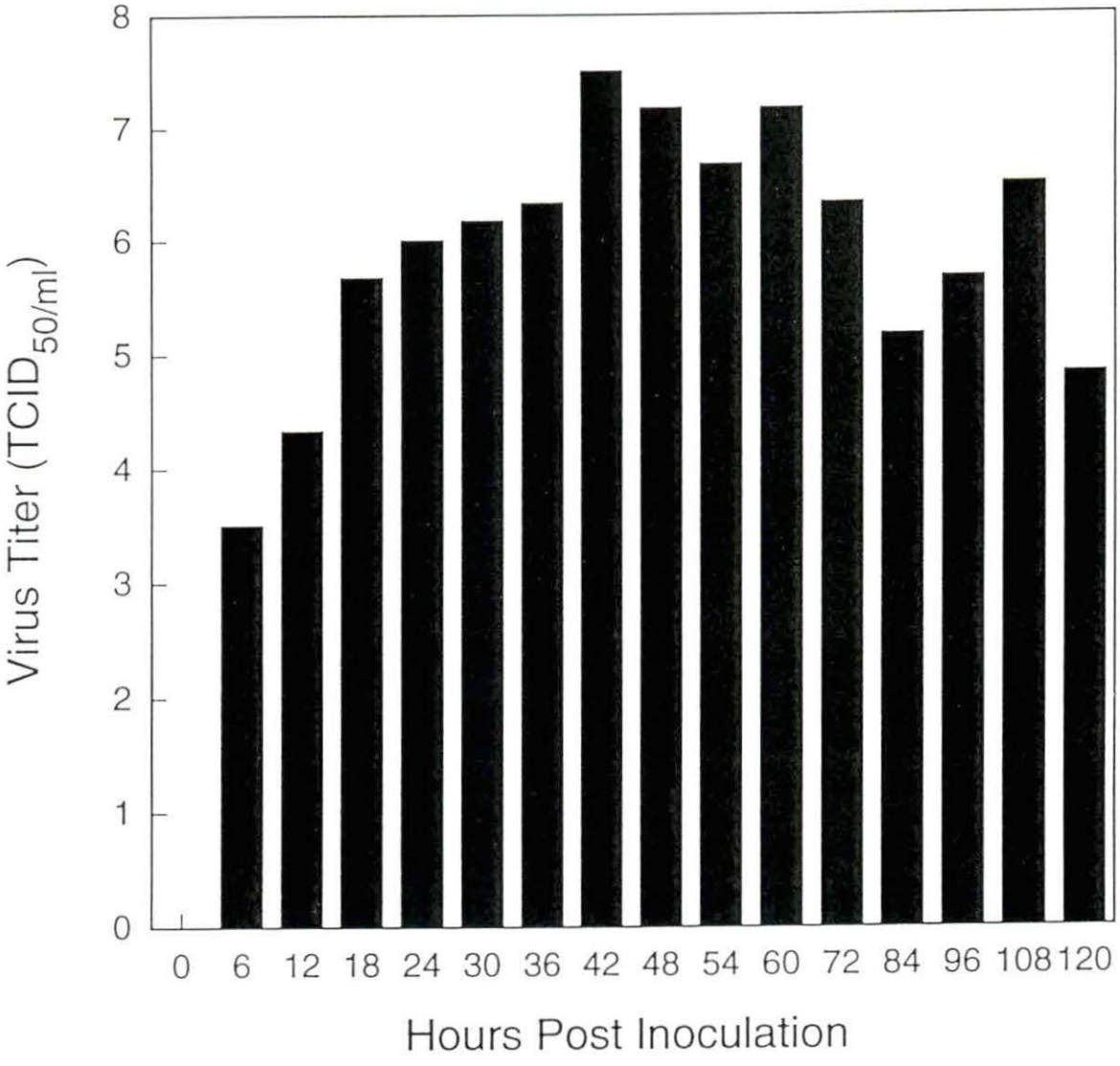


Figure 4. The growth of porcine paramyxovirus on a PSP-27 cell monolayer.

did not inhibit PPMV-induced CPE on PSP-27 cells when tested at a dilution of 1:8.

Pathogenicity of Cell Culture Propagated Porcine Paramyxovirus for Pigs

The six SPF pigs inoculated with cell-culture-propagated PPMV had slightly increased respiratory rates beginning at 1 DPI that continued through 10 DPI when compared to the uninoculated control pigs. There was no significant difference in rectal temperatures between principal and control pigs. Both principal and control pigs remained vigorous and retained their appetites throughout the trial. One principal pig died on the fifth DPI.

No gross lesions were observed in the euthanized principal or control pigs. A gastric ulcer was present in the principal pig that died on the fifth DPI. No other gross lesions were noted in this pig.

Microscopically, the lungs of all principal and control pigs were normal. The alveolar septa were of normal thickness and the epithelium lining bronchioles were intact. A severe focal vasculitis with locally extensive gliosis was noted in the brain stem of the PPMV-inoculated pig that died at 5 DPI (Figure 5).

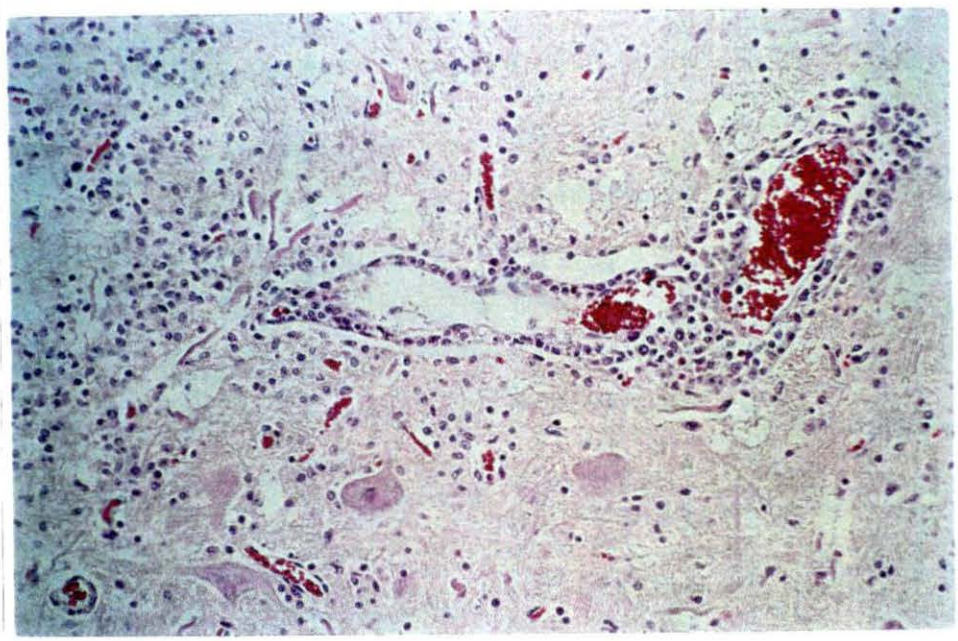


Figure 5. Severe focal vasculitis with locally extensive gliosis in the brain stem of a specific pathogen free pig at 5 days following infection with porcine paramyxovirus.

Antibodies in Sera of Pigs Inoculated with Porcine Paramyxovirus

Antibodies against PPMV were not detected in serum samples collected from principal or control pigs prior to intranasal inoculation, at 3 DPI, or 5 DPI. Three of the principal pigs had a serum titer against PPMV of 1:2 at 7 DPI. The fourth principal pig had not seroconverted at 7 DPI. By 10 DPI, all four principal pigs had seroconverted with antibody titers of 1:8, 1:16, 1:8, and 1:8. Antibodies against PPMV were detected in serum samples collected from principal pigs for the remainder of the trial which was completed at 24 weeks PI. During this time period anti-PPMV serum antibody titers of all principal pigs ranged between 1:8 and 1:64. The time (DPI) of serum sample collection had a significant affect ($P<.01$) on the level of serum antibodies against PPMV. A graph of serum antibody titers to PPMV in serum samples collected from inoculated SPF pigs is provided in Figure 6. Control pigs did not develop antibodies against PPMV. Serum antibody titers against PPMV differed significantly ($P<.05$) between control and PPMV-infected pigs. However, no significant differences ($P>0.05$) in virus titers were noted among replications of serum samples collected from SPF pigs infected with PPMV when an analysis of variance was

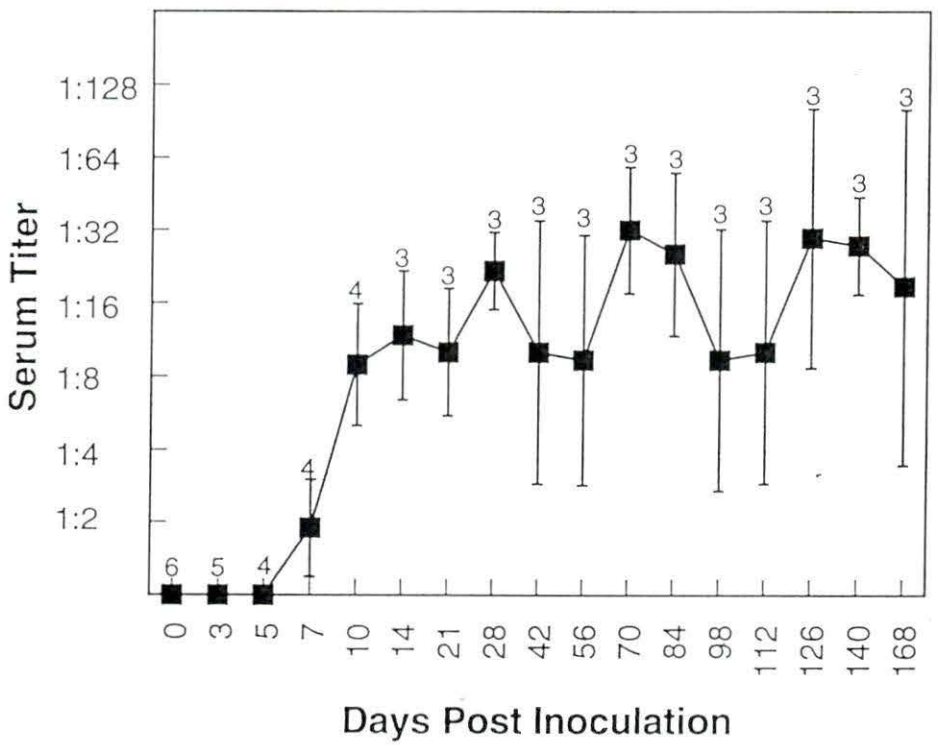


Figure 6. Specific pathogen free pig serum antibody titers to porcine paramyxovirus.

performed using the general linear models procedure in the Statistical Analysis System software.

Determination of Seroprevalence in Iowa's Swine Population

Six of the 876 swine serum samples tested inhibited PPMV-induced CPE, and were considered positive for antibodies to PPMV. These six serum samples were collected from 5 different farms participating in the NAHMS study. Nine of the 876 serum samples were retested because they were suspected positives at the 1:8 dilution. These serum samples were diluted and tested at 1:2, 1:4 and 1:8. Two of these samples were positive at a dilution of 1:2, one was positive at a dilution of 1:4 and one was positive up to a dilution of 1:8.

Twenty three serum samples required retesting due to contamination with bacteria. Additional allotments of each serum sample were heat inactivated and titer determinations were repeated. Of these 23 serum samples: 11 samples were still contaminated, 2 tested positive, and the additional 10 did not show the presence of antibodies against PPMV.

DISCUSSION

In this study a method for the cultivation of a porcine paramyxovirus isolate (ISU-92) was developed and standardized. Electron microscopy and serologic testing (Janke et al., 1992) were used to confirm that this isolate did indeed belong to the family Paramyxoviridae. A virus neutralization test was developed for the detection of serum antibodies directed against PPMV. To determine the pathogenicity of this isolate, six 6-week-old SPF pigs were inoculated intranasally with cell-culture-propagated PPMV. Although clinical signs of disease were very mild in PPMV-inoculated pigs, anti-PPMV antibodies were detected as early as 7 DPI, and persisted for 24 weeks PI. The neutralization test was used on serum samples from a NAHMS study to determine the seroprevalence of PPMV in Iowa swine herds.

Several cell lines have been used to support the growth of paramyxoviruses. MDBK cells have been used to propagate Newcastle disease virus (King, 1993) and Simian virus 5 (Sheshberadaran and Lamb, 1990). Primary monkey kidney and Vero cells have been used to support the growth of human parainfluenza type 2 virus isolates (Tsurdome et al., 1989). Blue eye pig disease virus has been grown on PK 15 (Stephano et al., 1988) and porcine IBRS cells (Berg et al., 1992).

Canine distemper virus has been grown on Vero cells (Appel et al., 1991). Primary rhesus monkey kidney cells are the standard for isolating mumps virus from suspected patient specimens (Wolinsky and Waxham, 1990).

After evaluating five cell lines to support the growth of isolate ISU-92, the PSP-27 cell line was chosen. Other cell lines evaluated may have supported viral growth, but were not pursued beyond the third passage. ST cells were the exception in which a total of eighteen viral passages were made. The decision to propagate PPMV using the PSP-27 cell line was based on the observation of a visible CPE with the third viral passage. This CPE included the formation of syncytia or multinucleated giant cells, followed by cell lysis. The CPE observed in monolayers of PPMV-infected PSP-27 and FPK cells were very similar to those reported in tissue cultures and cell lines infected with other members of the genus Paramyxovirus. Henle et al. (1954) described mumps virus induced CPE in tissue cultures as "large homogeneous masses of cytoplasmic enclosing numerous nuclei". Syncytia formation have been reported in PK 15 cell monolayers infected with the causative agent of swine blue eye disease (Stephano et al., 1988), Vero cell monolayers infected with canine parainfluenza virus (Baumgartner et al., 1981), and in the epithelial cells

of alveoli and bronchioles of PI3 virus infected cattle (Tsai and Thomson, 1975).

Janke et al. (1992) showed that ISU-92 was antigenically related to parainfluenza 1, 3, and 4B viruses by immunofluorescence. Examination of the physical appearance of ISU-92 by electron microscopy provided additional support that it was indeed a member of the family Paramyxoviridae. The PPMV particle presented in Figure 1 has a morphology characteristic of paramyxoviruses. Virions were pleomorphic and varied in size, ranging from 150 to 250 nm in diameters. Their morphology was consistent with descriptions of other members of the family Paramyxoviridae (Kingsbury, 1990). Filamentous forms, as described by Lipkind et al., (1986), were also observed. Glycoprotein complexes, or "spikes", which are characteristic of enveloped negative stranded viruses (Kingsbury et al., 1978), were visible on the surface.

The virus neutralization test provided an efficient means for detection of antibodies to PPMV. With this test the CPE induced in PSP-27 cells by PPMV easily distinguished infected from noninfected cell monolayers. Results obtained using the VN test were repeatable. No significant differences ($P>0.05$) in virus titers were noted among replications of serum samples collected from SPF pigs infected with PPMV when analyzed using Statistical Analysis System software.

Although the virus neutralization test was chosen as the test method in this study, fluorescent antibody and immunoperoxidase tests should not be ruled out as alternative testing procedures. The FA and immunoperoxidase tests were not evaluated on the PPMV infected PSP-27 cell line. These alternative tests may be preferred by veterinary diagnostic laboratories that use them routinely for the detection of antibodies against other viral agents.

Cell-culture-propagated PPMV (isolate ISU-92) was only mildly pathogenic for 6-week-old SPF pigs. Clinical signs in experimentally infected pigs bore little resemblance to the wide range of respiratory distress and CNS disorders observed in pigs in the field case from which the virus was isolated. The only lesion observed in experimentally infected pigs was vasculitis in the brain of one pig, which was consistent with lesions observed in PPMV-infected field case pigs.

There are several possible explanations for the reduced severity of clinical disease, and gross and microscopic lesions in PPMV-infected research pigs compared to PPMV-infected field case pigs. In the experimental trial the route of inoculation may not have provided sufficient exposure of challenge virus to susceptible tissues. The virus titer of the inoculum may have been lower than the virus titer with which pigs were challenged in the field case. The virus also

may have been attenuated by serial passage in cell culture making it unable to reproduce the severity of clinical disease seen in field case pigs. Several reports on paramyxovirus isolates describe passage in cell or tissue culture that reduce the virulence of the isolates for experimentally infected animals (Reisinger et al., 1959; Betts et al., 1964; Dawson et al., 1964; Appel and Gillespie, 1972). The experimental setting either eliminated or greatly reduced stress factors that are present in a typical swine operation. Trial pigs were not subjected to daily fluctuations in environmental temperature or humidity. Immoderate pig density and exposure to incoming pigs as seen in some continuous flow buildings were not factors affecting trial pigs. Such factors could enhance the severity of the disease and may have contributed to differences in mortality seen between the two finishing buildings in the field case. Death loss was highest in the first finishing barn which was managed as a continuous flow building. No death loss occurred in the finishing building that was managed as an all-in, all-out facility during the epizootic (Janke et al., 1992).

PPMV was the only virus identified in tissues collected from the field case pigs. Other viruses may have contributed to the severity of disease, but were undetected by laboratory examination. Bronchiolar epithelial necrosis was observed in

the lungs of field case pigs but was not reproduced in experimental trial pigs. This may suggest involvement of an additional viral agent in the field case pigs, such as SIV.

Serum antibody titers against PPMV were first detected in principal pigs in the SPF trial at 7 DPI. The appearance of antibody at this time is characteristic of a primary immune response (Tizard, 1987). The anti-PPMV antibodies in principal pigs in the SPF pig trial did not exceed a titer of 1:64 throughout the duration of the trial. Although antibody titers remained relatively low, they were detectable at 24 weeks PI, with an average titer of 1:32. The long serum antibody half-life against PPMV is advantageous from a diagnostic standpoint as it allows a generous lag time between PPMV outbreaks and serum sample collection for the identification of PPMV infected herds.

Twenty three of the 876 NAHMS serum samples tested were contaminated with bacteria. These samples were retested to rule out the possibility of a break in sterile technique while conducting the virus neutralization test. Ideally, the serum samples would have been filtered prior to retesting; however there was an insufficient quantity of serum to allow filtration. Two of the repeated samples contained PPMV neutralizing antibodies with titers of 1:4 and 1:8. Eleven of the retested samples were again contaminated on the repeated

tests. Therefore, the presence or absence of PPMV neutralizing antibodies in these 11 samples remains undetermined. The remaining 10 samples were negative.

Only six of the serum samples representing 5 swine herds in the NAHMS study had antibodies against PPMV. Such a low incidence of PPMV infection was suprising. One possible explanation is that PPMV infection was low at the time NAHMS samples were collected from swine in 1988 and 1989. The field case outbreak from which PPMV was isolated did not occur until November of 1991. It is possible that PPMV is now more widespread than data collected from the testing of NAHMS serum samples would suggest. Another possibility is that these samples represent false positive reactions. This is highly unlikely, as experimental data has shown none of the control samples and sera against other selected swine viruses to be seropositive for PPMV.

The source of PPMV infections remains obscure. Isolate ISU-92 reacted with antiserum against parainfluenza 1, 3, and 4B viruses. The possibility exists that field case pigs may have served as aberrant hosts for a human parainfluenza virus or bovine PI3 virus infection.

CONCLUSIONS

A virus isolate, designated ISU-92, was recovered from a field case of pigs exhibiting signs of respiratory distress and CNS disorders. This isolate was determined to be a member of the genus Paramyxovirus by reacting to antisera against parainfluenza 1, 3 and 4B viruses (Janke et al., 1992). A distinct CPE characterized by multinucleated giant cells is produced by PPMV inoculation onto PSP-27 cell line that is very similar to those reported in tissue cultures and cell lines infected with other members of the genus Paramyxovirus. Examination of the physical appearance of ISU-92 by transmission electron microscopy provided additional support that it was indeed a member of the family Paramyxoviridae.

A virus neutralization test was developed for the detection of serum antibodies against PPMV. The virus neutralization test is repeatable. When serum antibody titers against PPMV were determined in triplicate using 60 serum samples collected from PPMV-infected-SPF pigs, statistical analysis revealed no significant difference ($P>.05$) among the three replications. Antibodies against PPMV were detected in the serum of SPF pigs experimentally infected with PPMV by 7 DPI, and remained at detectable levels for the duration of the trial (24 weeks PI).

NAHMS serum samples collected from 36 swine farms in 1988 and 1989 were tested for the presence of antibodies against PPMV. Of the 876 serum samples tested, only six (representing 5 swine herds) had anti-PPMV antibody titers. This low number of positive serum samples may reflect a low level of PPMV infection in 1988 and 1989. Although the seroprevalence of PPMV is believed to be very low, more recent swine serum samples should be tested for the presence of PPMV neutralizing antibodies.

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APPENDIX

Specific Pathogen Free Pig Serum Antibody Titers to Porcine Paramyxovirus.

PPMV-Inoculated Pig Number	DPI	Titer (average of 3 VN test)
620	0	0
620	3	0
620	5	0
620	7	0
620	10	1:8
621	0	0
622	0	0
622	3	0
622	5	0
622	7	1:2
622	10	1:16
622	14	1:32
622	21	1:16
622	28	1:16
622	42	1:16
622	56	1:16
622	70	1:32
622	84	1:32
622	98	1:32
622	112	1:32
622	126	1:64
622	140	1:16
622	168	1:64
623	0	0
623	3	0
623	5	0
623	7	1:2
623	10	1:8
623	14	1:8
623	21	1:8
623	28	1:32
623	42	1:32
623	56	1:32
623	70	1:16
623	84	1:8
623	98	1:16

Specific Pathogen Free Pig Serum Antibody Titers to Porcine Paramyxovirus Continued.

PPMV-Inoculated Pig Number	DPI	Titer (average of 3 VN test)
623	112	1:16
623	126	1:32
623	140	1:32
623	168	1:64
624	0	0
624	3	0
624	5	0
624	7	1:2
624	10	1:8
624	14	1:8
624	21	1:8
624	28	1:32
624	42	1:2
624	56	1:2
624	70	1:64
624	84	1:32
623	98	1:2
624	112	1:2
624	126	1:64
624	140	1:32
624	168	1:2
625	0	0
625	3	0

Control Pig Number	DPI	Serum Titer
648	0	0
648	3	0
648	5	0
648	7	0
648	10	0
649	0	0
649	3	0
649	5	0
649	7	0
649	10	0
650	0	0
650	3	0