INFECTION AND IMMUNITY, Oct. 1974, p. 718-723

Vol. 10, No. 4
Printed in U.S.A.

Porcine Parvovirus: Natural and Experimental Infections of the Porcine Fetus and Prevalence In Mature Swine[1]

D. R. REDMAN, E. H. BOHL, AND L. C. FERGUSON

Department of Veterinary Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Received for publication 12 August 1974

Antibodies against porcine parvovirus were detected in 17 of 116 prenursing pig sera. Antibodies against transmissible gastroenteritis or ECPO-6 (an enterovirus) were not detected in prenursing sera of the pigs tested. Seventy-seven percent of 129 serum samples from 23 Ohio farms and 82% of 96 samples from slaughter plants in Ohio were serologically positive for porcine parvovirus. Mummies or other abnormalities were not observed in newly born pigs exposed to porcine parvovirus by the transuterine route 101 days after gestation. Indirect evidence suggested that the virus had not spread to other fetuses following exposure after 101 days at least not in a sufficient amount of time to stimulate detectable antibody. Direct intrafetal exposure to porcine parvovirus (i.m. injection, transutero) after 62 days of gestation resulted in dealth and mummification of the two fetuses, and apparently in the subsequent spread of the virus, as five of nine live pigs born were serologically positive for porcine parvovirus and these five pigs had not been injected with the virus. Immunoglobulin G was detected in all newborn pigs irregardless of known antigenic stimulation or the presence of specific antibody. In general, the presence of immunoglobulin M or immunoglobulin A in fetal serum was correlated with a history of antigenic stimulation or the presence of detectable antibody.

Various viral agents including porcine enteroviruses (7), the viruses of hog cholera (6, 8), pseudorabies (9, 10), Japanese encephalitis-B virus (17), Japanese hemagglutinating virus (19), and parvovirus (3, 4) have been shown to cause fetal infection resulting in stillbirths, malformations, embryonic death, or infertility.

Cartwright et al. (3) reported isolating viral agents from herds with problems of infertility, abortions, and stillbirths in pigs. The majority of the isolates were of the same type, designated FS59e/63. Subsequent studies on the physical and biological properties of the prototype virus suggest that it is a member of the picorna or parvovirus group (4, 5). Johnson et al. (12, 14) have also reported isolating parvovirus from aborted, stillborn, and normal pigs. Morimoto (18, 19) recently reported isolating parvoviruses from brain tissue of stillborn pigs in Japan. The reported (3) isolation of this virus from semen suggested the possibility that service by infected boars may contribute to the occurrence of repeat breeders.

Bachman and Mayr (1, 15) have reported isolating porcine parvovirus (PPV) in tissue cultures from the kidneys of conventionally reared, healthy 2- to 5-week-old pigs. The first reported isolation of PPV from swine in the United States was by Mengeling (16) and that was from the nasal turbinates. Mengeling (16) did suggest the possibility of fetal infection with PPV based on serologic studies with prenursing serum. In the course of studying the response of fetal swine to ECPO-6 or transmissible gastroenteritis (TGE) virus, it became apparent that PPV was probably involved in natural infections of the porcine fetus.

The purpose of the present report is to describe our experience with PPV in respect to natural fetal infections, experimental exposure of the fetus, immune response of the fetus, and prevalence of PPV infection in Ohio swine.

MATERIALS AND METHODS

Fetal exposure with PPV. Two sows were subjected to laparotomy, and two fetuses of each sow were injected by the transuterine route with PPV. Fluid (0.1 ml) from a virus pool [designated PPV(14PK-11/71)] with a hemagglutination (HA) titer of 320 was injected in each fetus. (The original PPV virus used in these studies was kindly furnished by Ben Sheffy, Cornell Univ., Ithaca, N.Y., under Federal permit.) Sterile cell culture medium (0.1 ml) was used to inject two litter mates. Prior to use, all material (virus and media) used for injecting fetuses

[1] Journal Article 59-74 of the Ohio Agricultural Research and Development Center, Wooster, Ohio.

was passed through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.), and fetuses were injected directly into the biceps femoris muscle using a tuberculin syringe with a 23-gauge 1-inch needle. The sows were housed in an isolation room for the duration of the pregnancies which were uneventful.

Identification of injected fetuses. Trypan blue (2.5% suspension) that had been autoclaved and filtered (0.45-μm membrane) was used to identify the injected fetuses. The dye was injected subcutaneously in one of various locations of the fetus, such as legs, base of tail, and ears, for reference points.

Cell cultures. Cultures of porcine kidney cells were grown in 4-ounce prescription bottles. The procedures were similar to those described by Hancock et al. (11). The growth medium consisted of 0.5% lactalbumin hydrolysate in Hanks balanced salt solution, 10% inactivated bovine serum, and antibiotics (100 U of penicillin, 100 μg of streptomycin, and 25 U of mycostatin per ml). The cell cultures were used for serum neutralization tests and virus isolation procedures. Cell cultures were occasionally stained with a fluorescein-conjugated PPV antiserum obtained from gnotobiotic pigs in an attempt to identify PPV in suspected material.

Serum neutralization. The serum neutralization test was used to determine serum antibody titers for ECPO-6 and TGE viruses. The test involved the neutralization of a determined amount of ECPO-6 or TGE virus by varying dilutions of heat-inactivated serum. Neutralizing antibody titers are reported as the reciprocal of the serum dilution, which resulted in a plaque reduction of 80%. Titers of less than 1 are reported as negative. The technique of conducting the plaque-reduction test for determining neutralizing antibody against TGE has recently been reported (2).

Hemagglutination inhibition (HI). Serum samples to be tested for PPV antibodies were heat-inactivated at 56 C for 30 min, then treated with kaolin and guinea pig erythrocytes to remove nonspecific inhibitors (16). After treatment, the supernatant was harvested for use in the HI test. The original serum was calculated to have been diluted 10-fold during the treatment.

Doubling dilutions of the treated serum were made in 0.2-ml volumes. To each tube was added 0.2 ml of the parvovirus antigen (containing 4 HA units). After incubating for 30 min at room temperature, 0.4 ml of a 0.5% suspension of guinea pig erythrocytes was added. Appropriate serum, saline, and virus controls were examined similarly. The contents were mixed, incubated at room temperature for 2 to 3 h, and examined for inhibition of HA.

The reciprocal of the highest dilution of serum eliciting a complete HI response was recorded as the end point. Sera that were negative in the 1:10 dilution are reported as negative.

Gel filtration. Selected fetal or newborn pig serum samples (2 to 3 ml) were passed through a system of 2 columns in a series. The columns (2.5 by 45.0 cm) were packed with Sephadex G-200 (Pharmacia, Upsala, Sweden). A flow rate of 16 ml/h was maintained by a peristaltic pump (Buchler Instruments, Fort Lee, N.J.). The buffer system used was 0.1 M tris (hydroxymethyl) aminomethane (Fisher Scientific Co., Fairlawn, N.J.) in 0.2 M NaCl, adjusted to pH 8 with HCl.

The eluate was collected in fractions of 3 ml in each tube and the optical density at 280 nm was determined in a Gilford model 240 spectrophotometer. The optical density values were plotted against tube numbers. The G-200 chromatograms illustrated do not include the fractions preceding the void volume. Portions of the eluate from the tubes were examined by immunodiffusion techniques for presence of immunoglobulins and were tested for the presence of neutralizing or HI antibodies.

Immunodiffusion. Serum samples and Sephadex G-200 fractions of serum were tested for the presence of immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG) by the double microimmunodiffusion technique. This procedure is a slight modification of the Ouchterlony method described by Wadsworth (21). The technique utilized 1% Noble agar (Difco, Detroit, Mich.), 1% NaCl, and 1:10,000 thimerosal (merthiolate, E. Lilly & Co., Greenfield, Ind.). Monospecific rabbit antisera against porcine IgM, IgG, and IgA were utilized for immunoglobulin determination. The procedures for preparing the monospecific antisera have been reported (20).

RESULTS

Exposure of 101-day-old fetal pigs with PPV. Two fetuses of sow 22-10 were injected with PPV after 101 days of gestation, and two litter mates were injected with sterile medium. The sow farrowed 10 live, normal pigs after 112 days, 11 days postinjection. Pigs 1 and 2 (media-injected and pigs 3 and 4 (virus-injected) were readily identified by the blue dye. None of the pigs had nursed at the time of collection of specimens. The sow's HI titer for PPV at the time of exposure was 1,280, and at the time of farrowing it was 2,560. Serologically negative pregnant sows were not available at the time these studies were initiated.

Pigs 1 through 5 were sacrificed immediately, and tissues and blood were aseptically collected. The remaining five pigs (6 through 10) were returned to the sow. The kidneys from pigs 1, 2, and 5 were pooled and ground in sand, and a 10% suspension was made in tissue culture media. The kidneys from pigs 3 and 4 were pooled and treated in a similar manner. The fluids from the kidney tissues were inoculated onto young porcine kidney cell sheets in an attempt to re-isolate the parvovirus. After several attempts and passages, no HA activity or evidence of cytopathology were observed.

The immunologic results, at birth, from the sow and the five pigs examined are shown in Table 1. HI antibodies were not detected in the serum of the two medium-injected pigs (1 and 2); in contrast, HI titers of 1,280 and 320 were present in the sera of the virus-injected pigs (3

and 4). Pigs 2 and 3 were adjacent in the uterus.

Although the dam had a serum neutralization titer of 18 against ECPO-6 virus, antibodies against this virus were not detected in the sera of pigs 2, 3, and 4. Sera of pigs 1, 3, 4, and 5 were tested for antibodies against TGE and all were negative, even though the sow had a titer of 64 against TGE virus.

Sephadex G-200 chromatogram of the serum of pig 3 is presented in Fig. 1. The HI titers of the fractions ranged from 2 to 256. IgG and IgM were detected in the serum of this animal. The 3 fractions, representing the first peak, contained IgM and the next 3 contained IgG (Fig. 1). The fractions with IgM present were also the ones with the highest antibody titers for PPV. IgA was not present in the serum or any of the fractions of pig 3.

TABLE 1. *Immunologic results after injecting two 101-day-old fetuses with parvovirus*[a]

Animal[b]	Antibody titers[c]			Ig class		
	PPV	TGE	ECPO-6	M	G	A
22-10	1,280	ND	ND			
22-10	2,560	64	18			
1	—	—	ND	–	+	–
2	—	ND	—	–	+	–
3	1,280	—	—	+	+	–
4	320	—	—	+	+	–
5	—	—	ND	–	+	–

[a] Sow farrowed 11 days after fetal injection.

[b] 22-10, Sow at the time of fetal injection and sow at the time of farrowing, respectively. Pigs 1 and 2 were diluent injected and pigs 3 and 4 were virus injected.

[c] For TGE and ECPO-6, the minus indicates no antibody detected at 1:1 dilution; for PPV, it indicates no antibody detected at 1:10 dilution. Serum antibody of piglets was precolostral. ND, Not done.

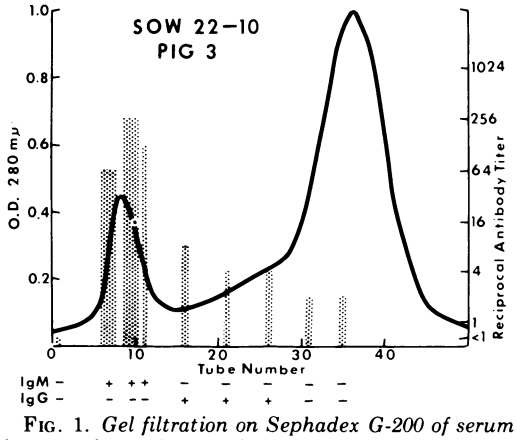

FIG. 1. *Gel filtration on Sephadex G-200 of serum from newborn pig (112 days) injected 11 days previously with PPV. Antibody titer of 1,280 for PPV. PPV antibody titers are represented by dotted bars, and classes of immunoglobulins in selected unconcentrated eluate fractions are shown. IgM and IgG were present in the serum. IgA was not present in the serum or fractions.*

The chromatogram profile for pig 1 (noninfected and serologically negative) was essentially the same as that for pig 3. All the fractions tested were negative for immunoglobulins. The sera of all five pigs tested contained IgG but not IgA. IgM was found only in the serum of the two virus-injected pigs.

Exposure of 62-day-old fetal pigs. A second sow (0-0) was subjected to surgery after 62 days of gestation, and two fetuses were injected with 0.1 ml of PPV and 1 fetus with 0.1 ml of sterile medium. An HI titer of 2,560 was present in the serum of the sow at the time of surgery. A hysterectomy was performed after 113 days of gestation, or 51 days postinfection. At this time, the serologic titer of the sow for PPV was 1,280.

Nine live pigs and two mummies were obtained. One mummy was intact and the second one was disintegrating. Based upon crown-rump length, it was assumed the intact mummy died about 70 to 75 days after gestation.

The single diluent-injected pig was readily identified, based upon dye markings. Since evidence of trypan blue was not seen in the other living pigs, it was assumed the two mummies were the virus-injected fetuses.

Immunologic results on the serum samples examined are presented in Table 2. Of the nine live pigs present, four were serologically negative for PPV antibodies and five were positive. The titers in the positive specimens ranged from 640 to greater than 5,120.

Although the dam was serologically positive with a titer of 70 against ECPO-6 virus, the sera of eight pigs were tested and all were negative for ECPO-6 antibodies. Both the sow and five of the pigs' sera were negative for antibodies against TGE viruses.

The serum chromatogram for pig 1 is presented in Fig. 2. IgM, IgG, and IgA were present in the serum. None of the fractions revealed IgA based on immunodiffusion. IgG and IgM were present in certain of the fractions as shown in Fig. 2. The HI titers of the fractions ranged from 16 to >320 for PPV antibody. All three classes of immunoglobulins were detected in the sera of the five serologically positive pigs. In contrast, the four serologically negative pigs did not have IgA and one of the four did not have IgM.

Serologic survey for PPV antibodies in pigs. Of 116 prenursing serum samples from newborn piglets representing 52 litters, PPV

TABLE 2. *Immunologic results of injecting two 62-day-old porcine fetuses with parvovirus*[a]

Animal[b]	Antibody titer[c]		Ig class		
	PPV	ECPO-6	M	G	A
0-0	2,560				
0-0	1,280	70			
1	5,120	—	+	+	+
2 (mummy)					
3 (mummy)					
4	—	—	+	+	–
5	640	ND	+	+	+
6	5,120	—	+	+	+
7	—	—	+	+	–
8	—	—	–	+	–
9	1,280	—	+	+	+
10	—	—	+	+	–
11	5,120	—	+	+	+

[a] Pigs hysterectomy derived 51 days postinjection.

[b] 0-0, Sow at the time of fetal injection and sow at the time of farrowing, respectively. Pig 1 was diluent injected and pigs 2 and 3 were assumed to be the virus injected pigs.

[c] For ECPO-6, the minus indicates no antibody detected at 1:1 dilution; for PPV, it indicates no antibody detected at 1:10 dilution. Serum antibody titers of piglets were precolostral. ND, Not done.

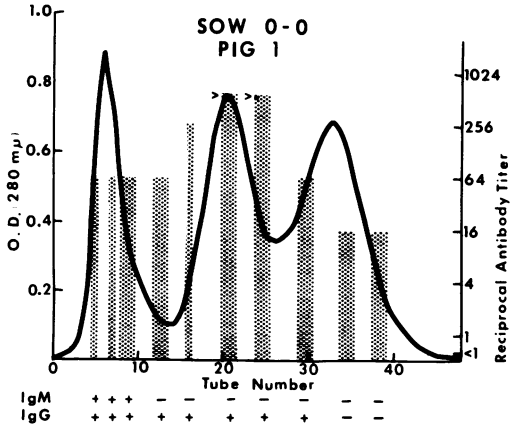

FIG. 2. *Gel filtration on Sephadex G-200 of serum from newborn hysterectomy-derived pig (113 days). Pig 1 was injected with medium, and two litter mates were injected with PPV 51 days previously. Pig 1 had an antibody titer of >5,120 for PPV. PPV antibody titers are represented by dotted bars, and classes of immunoglobulins in selected unconcentrated eluate fractions were shown. IgM, IgG, and IgA were present in the serum; IgA was not detected in any fractions.*

antibodies were present in 17 pigs from 6 different litters. Of these 17 pigs, 15 were positive for IgM and IgG by immunodiffusion, whereas 12 were positive for IgA. All 116 prenursing samples were negative for ECPO-6 antibodies and, of the 32 sera tested, all were negative for TGE. The dams of all 52 litters were serologically positive for ECPO-6 and PPV virus. Two of the six litters were from a herd that sporadically experienced reproductive problems, including mummies, weak pigs, and abnormally low rates of reproduction. The remaining four litters were from a herd in which reproductive problems have not been a complaint; however, occasional mummies were observed.

Serum samples from 23 swine farms throughout Ohio and from 5 packing plants were also tested. Of 129 samples from Ohio farms, 77% were positive for PPV antibodies. Of these 23 farms, 21 (91%) had animals in which part or all of the samples were positive for PPV antibodies. Titers were as high as 16,000. Of the 96 samples from 5 Ohio packing plants, 82% were positive for PPV.

Isolation of PPV from field cases. Once it was ascertained, based upon prenursing serology, that PPV might be involved in natural fetal infections, attempts to isolate the virus were initiated. Initially, this consisted of inoculating a 10% suspension of various tissues from selected cases (mummies, stillborn, and newborn weak pigs) onto established primary porcine kidney cell cultures. Observations were made for the development of cytopathic effect or HA activity. Blind passages were done after 10 to 20 days of observation. Selected specimens were examined by fluorescent-antibody techniques. At no time was there any suggestion of a viral agent being present by these procedures.

An agent suspected of being PPV was finally isolated after intensive efforts from one of three live pigs in a litter containing mummies and stillborn pigs. The prenursing serum of this pig was positive for PPV antibodies with a titer greater than 1:320. The PPV titer of the dam at the time of breeding was negative, 45 days later it was 1:81,920, and at 90 days postbreeding it was 1:10,240. Although the dam was serologically positive for ECPO-6, the prenursing serum of the pig was negative for antibodies to this virus. Attempts to isolate an agent on confluent porcine kidney cell cultures proved unsuccessful. However, feeding the same material to gnotobiotic pigs resulted in consistent seroconversion within 14 to 20 days. Subsequent attempts to isolate the agent in cell cultures from tissues of these exposed gnotobiotic pigs failed, yet 10% suspensions of these tissues resulted in seroconversion when fed to additional gnotobiotic pigs. HA activity suggestive of a viral agent being present was detected only after suspect tissues were trypsinized, washed, and grown in

vitro or after inoculating a 10% suspension of the suspect material onto primary 1- to 3-day-old (nonconfluent) porcine kidney cells. The same material inoculated onto confluent primary kidney monolayers did not result in detecting any HA activity. Cell cultures stained with fluorescein-conjugated PPV antiserum, 2 days after inoculation, revealed intense intranuclear fluorescence, suggesting infection with a PPV agent, whereas 4 to 5 days after inoculation, the fluorescence appeared more granular and the viral antigen was demonstrated in the cytoplasm.

DISCUSSION

Mummies or other abnormalities were not detected in pigs of sow 22-10 which had been intrafetally injected with PPV 101 days after gestation. In contrast, two mummified fetuses were found at term from the litter of sow 0-0 injected with the virus 62 days after gestation. Based upon the crown-rump length of the two mummies, it was ascertained they died sometime after fetal exposure. Since evidence of the trypan blue was not seen in the other living pigs, it was assumed the two mummies were the virus-injected fetuses.

PPV antibody was detected only in the two virus-injected fetuses of sow 22-10 and not from the medium-injected or noninjected litter mates. Indirect evidence would suggest that the virus had not spread to other pigs, at least with sufficient time to stimulate any detectable antibody. In the second case, however, where the pigs had been exposed for a much longer period of time, the virus apparently spread to litter mates, as five of the nine live pigs at birth were serologically positive for PPV, and these five pigs had not been injected with the virus.

Sows 22-10 and 0-0 were serologically positive for ECPO-6 virus, and sow 22-10 was positive for TGE virus. However, none of the pigs from these two sows had detectable antibodies against these two viruses. The information would suggest that transplacentally acquired maternal antibody was not present in the fetuses, either as a result of the surgery or the infection, and that the PPV antibody detected was probably of fetal origin. PPV-positive pregnant sows were used because we were unable to obtain serologically negative animals.

The data on the class of immunoglobulin detected in these two litters (Table 1 and 2) would suggest that infection for 11 days was not sufficient time for stimulation of the IgA system in the fetus, as IgA was not detected in the two virus-injected pigs of sow 22-10. It is possible that the IgA system may have been stimulated but was not yet providing detectable levels of systemic IgA. In contrast, IgA was present in all pigs serologically positive for PPV of sow 0-0, in which fetal exposure occurred 51 days previously. In addition to time, the discrepancy in finding IgA in one case and not the other might possibly be explained on the basis of the route of infection. The longer exposure period obviously provided additional routes (oral, intranasal, ocular) for infection other than direct intramuscular injection, which might affect the response of the IgA system.

Another interesting observation is that three pigs (4, 7, and 10) of sow 0-0 (Table 2) were serologically negative for PPV; however, IgM was detected in their sera on immunodiffusion. This apparent discrepancy of finding IgM but not PPV antibody may represent a very recent infection, and the immunodiffusion procedures may be more sensitive than HI techniques. It is also possible that the animal may have been exposed to an antigen not tested for.

Although not much is known regarding the importance of PPV in fetal infections of swine, the results of the serologic survey on newborn prenursing sera are in agreement with those of Cartwright et al. (5), Johnson (14), Mengeling (16), and Morimoto et al. (18), suggesting that this virus may infect the fetus, transplacentally resulting in fetal loss or reproductive problems. This would occur in gilts or sows initially exposed to PPV during pregnancy. In the present study, 17 of 116 prenursing sera were positive for PPV antibodies, yet all 116 were serologically negative for ECPO-6. This observation would suggest that PPV could be a major contributing factor in fetal loss or breeding problems of swine in the United States. Most of the reports regarding this virus have been from other countries (1, 4, 18), and only recently has there been a report of isolating PPV from swine in the United States (16).

Seventy-seven percent of 129 serum samples from 23 Ohio herds and 82% of 96 samples from slaughter plants in Ohio were serologically positive for PPV. Twenty-one of the farms had animals in which part or all of the samples were positive for PPV. This information indicates that PPV infection is common in the swine population. The history of the samples from the two herds, which did not have positive animals, is not known. Possibly the samples were from young breeding-age (6 to 8 months old) animals obtained before they entered the breeding herd. It has been our experience that most young gilts are negative for PPV antibodies at the time of entering the breeding herd and sero-convert

some time later. The serologic results reported in this study support those recently reported by Mengeling (16), who also suggested the widespread distribution of serologically positive animals in the swine population in the U.S.A.

The difficulty in isolating a PPV agent early in these studies may have been overcome if younger porcine kidney cells had been used. Recent experience in our laboratory with PPV has revealed that direct tissue culture of suspected material or inoculation of a 10% suspension of material onto very young (1 to 3 days old) porcine cells increased the appearance of HA activity. The agent finally isolated after passage through gnotobiotic pigs in all probability is PPV. Although not fully characterized, the fluorescence seen using monospecific PPV antisera is similar to that described by Mengeling (16). This experience in trying to isolate viral agents from suspected material reveals the potential value of gnotobiotic pigs for this procedure. It was our experience that the most sensitive means of detecting agents difficult to isolate was by use of the gnotobiotic animal resulting in sero conversion.

The implications of finding natural cases of fetal infections with PPV have recently been reviewed by Mengeling (16). Additional information is needed regarding the epidemiology and pathogenicity of PPV so that its significance to porcine fetal losses or reproductive problems in the United States may be ascertained.

ACKNOWLEDGMENTS

The technical assistance of Linda J. Saif, D. Huebner, and Mildred Simon in carrying out details of this work is gratefully acknowledged.

LITERATURE CITED

1. Bachman, P. A. 1969. Vorkommen and Verbreitung von picodna (parvo) Virus beim Schwein. Zentralbl. Veterinaermed. **13**:341-345.
2. Bohl, E. H., R. K. P. Gupta, M. V. F. Olquin, and L. Saif. 1972. Antibody responses in serum, colostrum, and milk of swine after infection or vaccination with transmissible gastroenteritis virus. Infect. Immunity **6**:289-301.
3. Cartwright, S. F., and R. A. Huck. 1967. Viruses isolated in association with herd infertility abortions and stillbirths in pigs. Vet. Rec. **81**:196-197.
4. Cartwright, S. F., M. Lucas, and R. A. Huck. 1969. A small haemagglutinating porcine DNA virus. I. Isolation and properties. J. Comp. Pathol. **79**:371-377.
5. Cartwright, S. F., M. Lucas, and R. A. Huck. 1971. A small haemagglutinating porcine DNA virus. II. Biological and serological studies. J. Comp. Pathol. **81**:145-155.
6. Dunne, H. W., and C. D. Clark. 1968. Embryonic death, fetal mummification, stillbirth, and neonatal death in pigs of gilts vaccinated with attenuated live virus hog cholera vaccine. Amer. J. Vet. Res. **29**:787-796.
7. Dunne, H. W., J. L. Gobble, J. F. Hokanson, D. C. Kradel, and G. R. Bubash. 1965. Porcine reproductive failure associated with newly identified SMEDI group of picorna viruses. Amer. J. Vet. Res. **26**:1284-1297.
8. Emerson, J. L., and A. L. Delez. 1965. Cerebellar hypoplasia hypomyelinogenesis and congenital tremors of pigs associated with prenatal hog cholera vaccination of sows. J. Amer. Vet. Med. Ass. **147**:47-54.
9. Gustafson, D. P. 1968. Some factors in the spread of pseudorabies virus among swine, p. 349-357. *In* Proc. 71st Annu. Meet., U.S. Livestock San. Ass. The Williams Printing Co., Richmond, Va.
10. Gustafson, D. P., J. R. Saunders, and R. M. Claflin. 1969. Effects of Aujeszky's disease in pregnant swine, p. 124-137. *In* Proceedings symposium on factors producing embryonic and fetal abnormalities in swine, no. 91-73. Agricultural Research Service, Hyattsville, Md.
11. Hancock, B. B., E. H. Bohl, and J. M. Birkeland. 1959. Swine kidney tissue cultures—susceptibility to viruses and use in isolation of enteric viruses of swine. Amer. J. Vet. Res. **20**:127-132.
12. Johnson, R. H. 1969. A search for parvoviridae. Vet. Rec. **84**:19-20.
13. Johnson, R. H. 1971. Transplacental infection of piglets with a porcine parvovirus. Res. Vet. Sci. **12**:570-572.
14. Johnson, R. H., and D. F. Collings. 1969. Experimental infection of piglets and pregnant gilts with a parvo virus. Vet. Rec. **85**:446-447.
15. Mayr, A., P. A. Bachmann, G. Siegel, H. Mahnel, and B. E. Sheffey. 1968. Characterization of a small porcine DNA virus. Arch. Gesamte Virusforsch. **25**:38-51.
16. Mengeling, W. L. 1972. Porcine parvovirus: properties and prevalence of a strain isolated in the United States. Amer. J. Vet. Res. **33**:2239-2248.
17. Morimoto, T. 1969. Epizootic swine stillbirth caused by Japanese encephalitis virus, p. 137-153. *In* Proceedings symposium on factors producing embryonic and fetal abnormalities, death, and abortion in swine, no. 91-73. Agricultural Research Service, Hyattsville, Md.
18. Morimoto, T., Y. Fujisaki, Y. Ito, and Y. Tanaka. 1972. Biological and physiochemical properties of porcine parvovirus recovered from stillborn piglets. Nat. Inst. Anim. Health. Quart. **12**:137-144.
19. Morimoto, T., H. Kurogi, Y. Miura, T. Sugimori, and Y. Fujisaki. 1972. Isolation of Japanese encephalitis virus and hemagglutinating DNA virus from the brain of stillborn piglets. Nat. Inst. Anim. Health Quart. **12**:127-136.
20. Saif, L. J., E. H. Bohl, and R. K. P. Gupta. 1972. Isolation of porcine immunoglobulins and determinations of the immunoglobulin classes of transmissible gastroenteritis viral antibodies. Infect. Immunity **6**:600-609.
21. Wadsworth, C. A. 1957. A slide microtechnique for the analysis of immune precipitates in gel. Int. Arch. Allergy Appl. Immunol. **10**:355-360.