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LESIONS RESULTING FROM INOCULATION OF PORCINE FOETUSES WITH PORCINE PARVOVIRUS

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ABSTRACT

PROZESKY, L., THOMSON, G. R., GAINARU, M. D., HERR, S. & KRITZINGER, L. J., 1980. Lesions resulting from inoculation of porcine foetuses with porcine parvovirus. *Onderstepoort Journal of Veterinary Research*, 269–274 (1980).

In utero inoculation of 15 sows at various stages of gestation with a local strain of porcine parvovirus (PPV) resulted in resorption, abortion or the birth of weak, dead, or mummified foetuses. Histopathological lesions observed in foetuses of sows slaughtered at various post-inoculation intervals consisted of a perivascular inflammatory reaction primarily observed in the brain and kidneys. The presence and extent of the inflammatory reaction were dependent upon the age of the foetus at the time of infection. In the sow a perivascular inflammatory reaction was found in the endometrium, while the larger blood-vessel walls were infiltrated by lymphocytes, and it is suggested that these vascular lesions may contribute to the reproductive failures associated with PPV.

Résumé

LÉSIONS DUES L'INOCULATION DE FOETUS PORCINS AVEC DU PARVOVIRUS PORCIN

L'inoculation in utero *de 15 truies, à des stades variés de la gestation avec une souche locale du parvovirus porcin (PPV) a résulté en résorption, en avortement ou en naissances de foetus faibles, morts ou mommifiés. Les lésions histopathologiques observées chez les foetus de truie abattues à des intervals variés après l'inoculation, consistaient d'une réaction inflammatoire périvasculaire d'abord observée dans le cerveau et les reins. La présence et l'intensité de la réaction inflammatoire étaient dépendantes de l'âge du foetus au moment de l'infection. Chez la truie une réaction inflammatoire perivasculaire fut trouvée dans l'endometrium, tandis que les parois des vaisseaux sanguins, plus grandes, étaient infiltrées par des lymphocytes et il est suggéré que ces lésions vasculaires puissent contribuer aux échecs reproductifs associés avec le PPV.*

INTRODUCTION

Porcine parvovirus (PPV) is a pervasive infection of pigs and has been incriminated as a cause of various reproductive disorders (Mengeling, 1975; Johnson, Donaldson-Wood, Joo & Allender, 1976; Thacker, 1978). Apart from the isolation of this virus from piglets with various ailments, PPV has also been isolated from normal piglets and foetuses (Joo & Johnson, 1976). Gross and histopathological lesions observed in foetuses infected with PPV at different stages of gestation have been reported (Narita, Inui, Kawakami, Kitamura & Maeda, 1974; Hogg, Lenghaus & Forman, 1977; Forman, Lenghaus, Hogg & Hale, 1977 and Lenghaus, Forman & Hale, 1978).

In South Africa, PPV was first isolated from foetuses during an outbreak of reproductive failure in a piggery (Pini, 1975), and since then it has been identified on a number of occasions as the cause of similar problems (Thomson, unpublished findings). These findings prompted an investigation into the effect of the local strain of PPV on the foetus and pregnant sow in order to compare our results with those already reported, with particular reference to the pathology.

MATERIALS AND METHODS

Source of virus inoculum

A PPV isolate from a still-born foetus submitted for diagnostic purposes was used at the 6th culture passage. Foetuses were inoculated with a stock containing $10^{2,7}$ culture infective doses/mℓ in piglet kidney cells and a haemagglutination (HA) titre of 1/160.

Cell cultures

Monolayer cultures of piglet kidney cells, shown to be free of PPV haemagglutinin, were prepared by conventional methods. For the isolation and titration of PPV, roller tube cultures, approximately 50% confluent, were inoculated with 10% tissue suspensions (0,2 mℓ/tube) and rolled for 2 hours at 37 °C. The tubes were washed with modified Eagle's medium (Glasgow) containing penicillin (0,6 mg/mℓ), streptomycin (1 mg/mℓ), neomycin (0,5 mg/mℓ) and Fungizone (8 mg/mℓ) both prior to and following the adsorption period, and finally incubated at 37 °C with the same medium containing 2% bovine serum. Cultures were examined every 2 days for the appearance of cytopathic effects. After 6 days the medium was poured off and replaced by 1,5 mℓ of serum-free medium, the tubes were frozen and thawed twice (−70/37 °C), and the medium checked for HA activity. Specific inhibition with a PPV antiserum of known titre was used to identify PPV as the cause of HA. Cultures which proved negative were passaged twice more in the same way before being regarded as negative.

Haemagglutination and haemagglutination inhibition tests

These tests were performed by a method described previously by Thomson, Mumford, Campbell, Griffiths & Clapham (1976), except that guinea-pig erythrocytes were used and all incubations were carried out at 4 °C. Non-specific effects of sera were removed by absorption with an equal volume of 25% kaolin in phosphate buffered saline, pH 7,2 (PBS) and being heated to 56 °C for 30 min.

Experimental animals and procedure

The sows were starved overnight and premedicated with azaperone* 20–30 min before the commencement of surgical procedures. After general anaesthesia with trichloroethylene** they were prepared for surgery in lateral recumbency and a blood sample was collected in a vacuum tube without preservatives or

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anticoagulant. In each case the uterus was exteriorized through the right lateral abdominal wall and 0,2 mℓ of the PPV preparation injected into the amniotic fluid of each foetus of one horn or intramuscularly, depending on the size of the foetus. The foetuses in the other horn were injected with 0,2 mℓ of PBS in the same manner and served as foetal controls. A silk suture, placed superficially in the uterine wall, marked the infected horn. The sows were inspected daily throughout the experiment. A total of 13 pregnant gilts, 6 of which were serologically positive and 6 negative, were infected *in utero* with PPV at different stages of gestation (Table 1). The pre-inoculation haemagglutination inhibition test (HI) titre of one sow was not available.

Foetuses of two gilts, one serologically positive and 1 negative, were inoculated intramuscularly or into the amniotic fluid with PBS as additional controls to determine the effect of the surgical procedures (Table 1). Sows which did not abort or farrow were slaughtered at set times (Table 1). Specimens of the uterus and a wide range of tissues from the foetuses were collected in 10% buffered formalin. For virus isolation each foetus was removed separately with its own placenta and the associated portion of the uterine wall. Foetal blood for antibody determination and tissues from aborted foetuses were collected in the same way. However, in 2 cases, the aborted foetuses were devoured by the sows and the only evidence of abortion was the presence of foetal and placental remnants in the pen. In the sows slaughtered, apart from the foetuses, only the uterus and placenta were examined histologically. Where the sows aborted, the placenta if available, was examined.

Formalin-fixed tissue was embedded in paraffin wax, sectioned at 3–5 μm and stained with haematoxylin and eosin (HE).

Results

Control sows

No foetal abnormalities were observed in the control sows or their litters that had been inoculated with PBS (Table 1).

TABLE 1 Results of foetal inoculation with PPV

Sow No.	Pre-innoculation HI titre	Size of litter	Number of foetuses inoculated with PPV	Number of foetuses inoculated with PBS	Gestational stage of foetus inoculated (days)	Method of inoculation	Results of inoculation
1	1/128 000	10	Not known	Not known	27	In amnoitic fluid	All foetuses were resorbed when sow was slaughtered 11 days after inoculation
2	< 1/200	15	Not known	Not known	31	In amnoitic fluid	All foetuses were resorbed when sow was slaughtered 3 days after inoculation.
3	Not available	5	4	1	41	Intramuscularly	All foetuses wer resorbed when sow was slaughtered 15 days after inoculation.
4	< 1/200	9	5	4	44	Intramuscularly	*Sow aborted 3 days after inoculation.
5	1/25 000	13	6	7	44	Intramuscularly	All foetuses were resorbed when the sow was slaughtered 11 days after inoculation.
6	1/51 200	15	11	4	47	Intramuscularly	All foetuses were resorbed when the sow was slaughtered 13 days after inoculation.
7	< 1/200	7	3	4	50	Intramuscularly	All foetuses were dead when the sow was slaughtered 3 days after inoculation
8	< 1/200	11	6	5	53	Intramuscularly	*Sow aborted 5 days after inoculation.
9	1/12 800	2	1	1	±60	Intramuscularly	*Sow aborted 5 days after inoculation.
10	1/12 800	4	3	1	71	Intramuscularly	Sow was slaughtered 22 days after inoculation. 3 foetuses were normal macroscopically and 1 died *in utero*.
11	< 1/200	6	2	4	79	Intramuscularly	Sow was slaughtered 11 days after inoculation. All the foetuses were intact.
12	< 1/200	7	4	3	80	Intramuscularly	Sow was slaughtered 11 days after inoculation. All the foetuses were intact
13	1/12 800	11	8	3	96	Intramuscularly	*Sow gave birth to weak, dead and one mummified foetus 10 days after inoculation
Controls							
14	1/51 200	9	Not known	—	34	In amniotic fluid	Foetuses were intact when the sow was slaughtered 14 days after inoculation
15	< 1/200	7	Not known	—	47	In amniotic fluid	Foetuses were intact when the sow was slaughtered 10 days after inoculation

* Sows which farrowed or aborted were not slaughtered

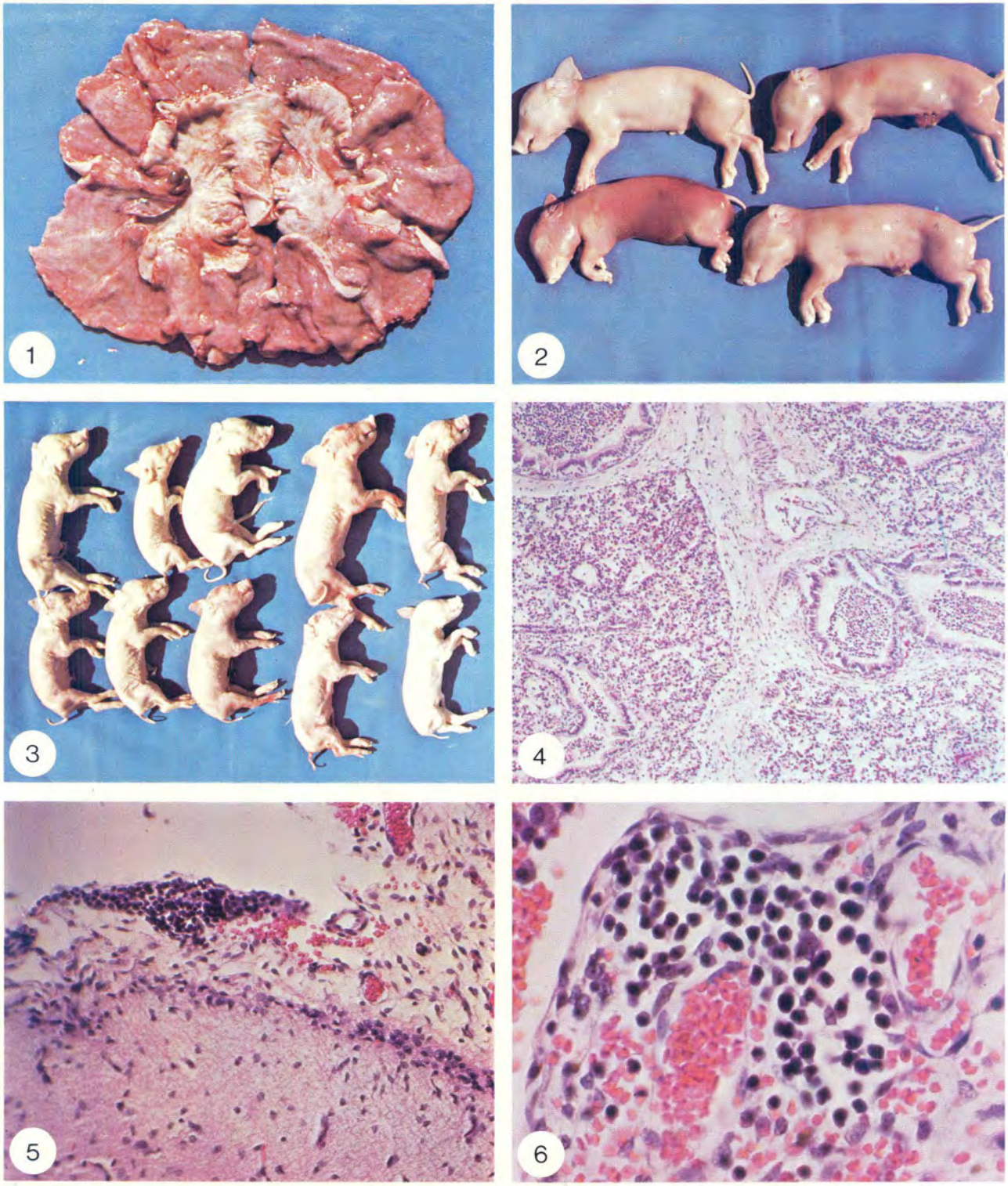
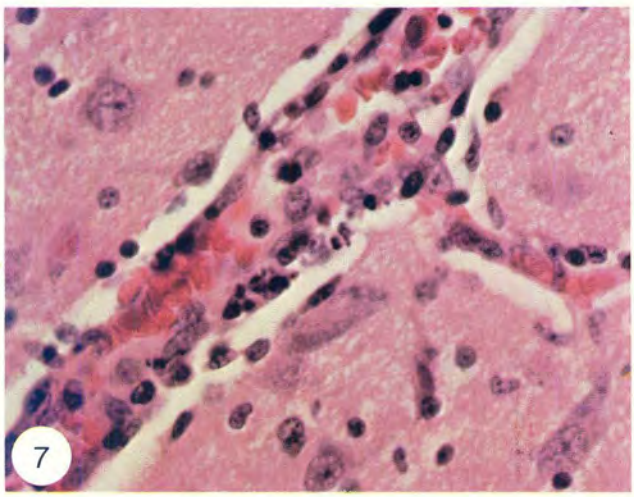
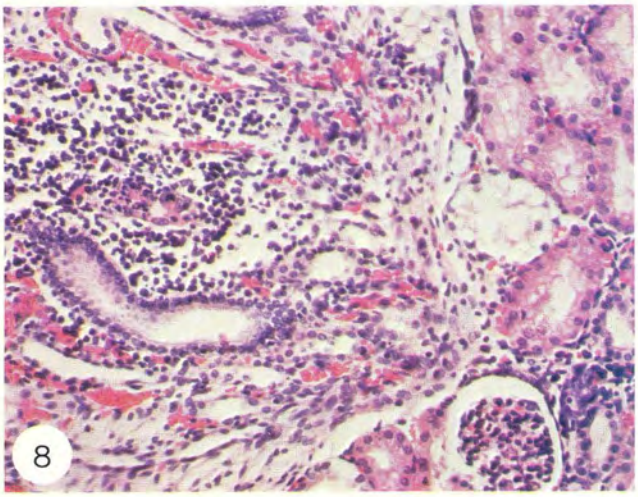
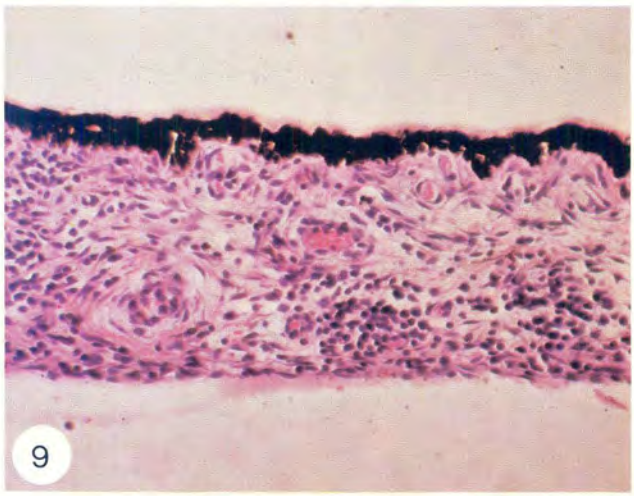
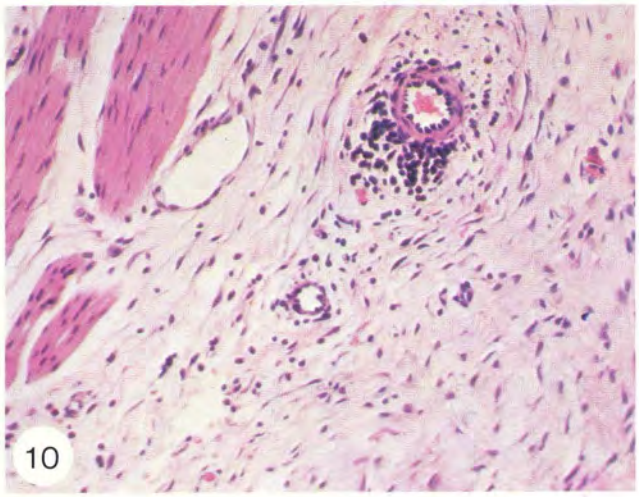


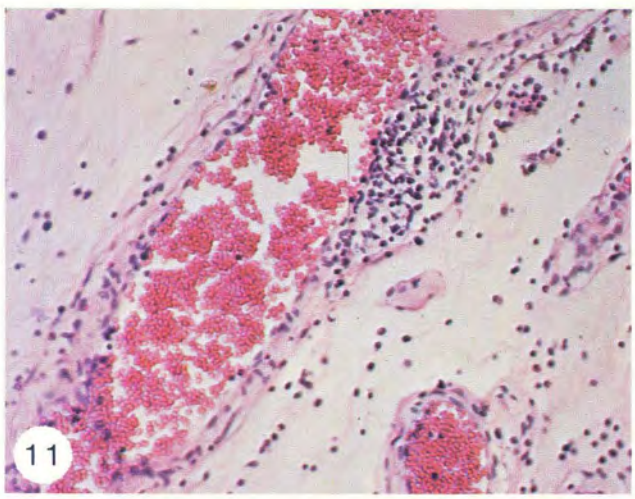
FIG. 1 Uterine horn of a sow inoculated with PPV at 41 days of gestation and slaughtered 15 days later. Foetuses resorbed
FIG. 2 Foetuses of Sow 10. They were inoculated at 71 days of gestation and the sow slaughtered 22 days later. The bottom left foetus died *in utero*
FIG. 3 Weak and still-born foetuses of a sow which farrowed 10 days after inoculation at 96 days gestation
FIG. 4 An accumulation of polymorphonuclear leucocytes in the bronchi of a foetus inoculated with PPV. He × 1200
FIG. 5 Vascular lesions in the meninges of a foetus. HE × 200
FIG. 6 Perivascular infiltration of round cells in the choroid plexus. HE × 1200











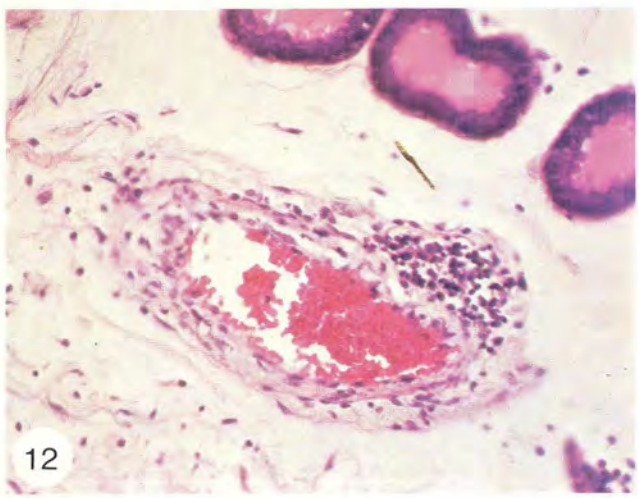


FIG. 7 A vasculitis in the brain substance. HE×1200
FIG. 8 Perivascular infiltration in the medulla and pelvic region of the kidney. HE×200
FIG. 9 Vascular lesions in the iris. HE×200
FIG. 10 Vascular lesions in the urinary bladder. HE×200
FIG. 11 Perivascular infiltration in the endometrium. HE×200
FIG. 12 Focal lymphocytic infiltration into a vein in the endometrium. HE×200

Experimentally infected sows

(i) *Resorption.*—Sows 1, 2, 3, 5 and 6 (Table 1), inoculated at 27, 31, 41, 44 and 47 days of gestation respectively, and slaughtered 11, 3, 15, 11 and 13 days later, resorbed the foetuses (Fig. 1).

(ii) *Abortion.*—Sows 4 and 8 (Table 1), in which the foetuses were infected at 44 and 53 days of gestation, aborted 3 and 5 days after infection, while Sow 9 aborted 5 days after the foetuses were infected. The exact date of service in the latter case was not known, but from the size of the foetus it was estimated that the sow was approximately 60 days pregnant.

(iii) *Fate of foetuses in sows slaughtered.*—Sows 7, 10, 11 and 12, infected at 50, 71, 79 and 80 days of pregnancy respectively, were slaughtered 3, 22, 11 and 11 days after infection (Table 1). All the foetuses of Sow 7 were dead, while only 1 foetus of Sow 10 died *in utero* (Fig. 2).

(iv) *Weak and still-born piglets.*—Sow 13, whose foetuses were infected at 96 days of gestation, gave birth 10 days after infection to 1 mummified foetus and 10 weak or dead piglets (Fig. 3). The weak piglets all died shortly after birth.

Gross pathology

A single foetus in Sow 11 showed a fibrinoid peritonitis and a slightly enlarged liver, but no gross abnormalities were observed in the other foetuses of this litter.

In 4 of the piglets from Sow 13 the lungs had a greyish, mottled appearance.

All foetuses that died *in utero* showed various stages of autolysis.

Foetal microscopic pathology

The most prominent microscopic lesion observed consisted of a perivascular infiltration of mononuclear cells (lymphocytes, lymphoblasts and plasma cells), accompanied by endothelial hypertrophy and hyperplasia of small bloodvessels. The perivascular inflammatory reaction was most prominent in 2 foetuses of Sow 10 infected at 71 days and in one each of Sows 11 and 12 infected at 79 and 80 days. The reaction was less conspicuous in 6 foetuses (3 each of Sows 11 and 12) and absent in 6 foetuses (1 of Sow 10, 3 of Sow 12 and 2 of Sow 11). No vascular lesions were found in the foetuses of Sow 13 infected at 96 days of gestation (Table 2).

Lungs

The lungs of 4 foetuses of Sow 13 showed an accumulation of polymorphonuclear leucocytes, necrotic cells and erythrocytes in the bronchi, bronchioles and alveoli (Fig. 4). The parenchyma was diffusely infiltrated with neutrophils, macrophages and a few eosinophils (Table 2). Pyknosis and karyorrhexis were prominent in some of these polymorphonuclear leucocytes. A striking feature was the presence of vacuoles in the hypertrophic alveolar epithelial cells which caused these cells to protrude into the alveolar lumen. Necrosis of the alveolar lining cells was occasionally observed. Lymphatics in the septa and those surrounding the larger bronchi were dilated and contained a few neutrophils.

Brain

Perivascular infiltrates were observed mainly in the meninges and choroid plexus (Fig. 5 & 6), while in foetuses with very severe lesions the inflammatory reaction accompanied blood-vessels even in the brain substance (Fig. 7). Macrophages containing haemosiderin pigment were occasionally seen near bloodvessels.

Kidneys

Perivascular infiltration was most prominent in the pelvic region of the medulla and pelvic loose connective tissue (Fig. 8). In foetuses where this reaction was pronounced, necrosis of tubular epithelial cells in the cortex accompanied by a lymphocytic and plasma cell infiltration was seen.

Liver

Liver lesions were found in only 2 foetuses, one each in Sows 11 and 13. These consisted of hypertrophic hepatocytes with vesicular nuclei, degenerative changes such as cloudy swelling, and hydropic and hyaline droplet degeneration. Bile stasis was observed in 1 of the 2 foetuses only.

Eyes

Vascular lesions as described above were seen in the iris (Fig. 9), ciliary body and choroid of 1 foetus (Sow 11). Eyes from 6 other foetuses examined were negative.

Lymphoreticular system

In infected foetuses, lymphocyte and lymphoblast proliferation around the central arteries in the spleen was a prominent feature, while reticulo-endothelial cell proliferation in the red pulp was observed. Follicular development in the bronchial and mesenteric lymph nodes with karyorrhexis in a few lymphocytes was prominent.

Heart and urinary bladder

Vascular lesions were seen in the hearts of 2 foetuses only (Sows 11 & 12). In those cases where the urinary bladder was examined the vascular lesions corresponded to those found in the kidneys of the same case (Fig. 10).

Maternal microscopic pathology

A similar perivascular infiltrate as described for the foetuses was an almost constant feature in the endometrium (Fig. 11). Four of the 5 uteri examined were affected, but a perivascular infiltrate was seen only occasionally in the placenta. Sows 1, 6, 11 and 12 showed this lesion, but it was absent in Sow 10. In addition the walls of the larger blood-vessels of the affected uteri, especially those of the larger veins, showed a focal or diffuse lymphocytic infiltration (Fig. 12).

In the sows in which resorption occurred, the amount of necrotic debris, macrophages and neutrophils in the uterine lumen depended on the size of the foetus at inoculation and on the time that elapsed after foetal death. This in turn influenced the extent of the mainly neutrophil reaction in the endometrium.

Virus isolation and serological results

Piglets

Of the 10 piglets (and 1 mummified foetus) born to Sow 13, the virus was isolated from 3 piglets and the mummified foetus. An HI titre was present in one of these virus-positive piglets and in 2 others (Table 2).

TABLE 2 Histological lesions

Sow No.	Size of litter	Stage of gestation (days) when foetuses were exposed to PPV	Day after inoculation when foetuses were autopsied	Number of foetuses	Perivascular infiltration			Pneumonia	Foetus inoculated with PPV	Foetal HI titre	PPV isolated from foetus
					Brain	Kidney	Lung				
10	4	71	22	1	++	++	—	—	Yes	1/1600	Yes
				2	++	++	—	—	Yes	1/1600	Yes
				3	—	—	—	—	No (Control)	1/800	Yes
				4	†	†	†	—	Yes	1/1600	Yes
11	6	79	11	1	+	—	—	—	Yes	1/320	Yes
				2	+++	+++	+	—	Yes	1/2560	Yes
				3	+	—	—	—	No (Control)	1/10	Yes
				4	+	++	—	—	No	1/10	Yes
				5	—	—	—	—	No	1/10	No
				6	—	—	—	—	No	1/10	No
12	7	80	11	1	+	—	—	—	Yes	1/640	Yes
				2	+	+	—	—	Yes	1/1280	Yes
				3	+	—	—	—	Yes	1/1280	Yes
				4	++	++	—	—	Yes	1/320	Yes
				5	—	—	—	—	No (Control)	Neg	Yes
				6	—	—	—	—	No (Control)	Insufficient serum for examination	No
				7	—	—	—	—	No (Control)	Neg	No
13	11	96	10	1	—	—	—	—	*	Neg	No
				2	—	—	—	++	*	1/20	No
				3	—	—	—	++	*	Neg	Yes
				4	—	—	—	—	*	1/20	No
				5	—	—	—	—	*	Neg	No
				6	—	—	—	—	*	Neg	No
				7	—	—	—	—	*	Neg	No
				8	—	—	—	—	*	Neg	No
				9	—	—	—	++	*	1/20	Yes
				10	—	—	—	++	*	Neg	Yes
				11	†	†	†	†	*	†	Yes

— = No lesions + = Mild lesions
++ = Moderate lesions +++ = Pronounced lesions
† = Not examined

* Litter of Sow 13 was born and therefore the non-infected piglets could not be identified

Aborted foetuses

No virus was isolated from the 2 foetuses of Sow 9 that aborted 5 days after infection nor were the sera examined for HI activity. The sera of the aborted foetuses from Sows 4 and 8 could not be examined for HI activity as the foetuses were devoured by the sows.

Foetuses from sows slaughtered

Virus was isolated from only one of the 3 foetuses inoculated in Sow 7 (Table 2). Because of advanced autolysis, no serum was collected for serological assays. In Sow 10, 3 out of the 4 foetuses were inoculated with the virus preparation and, of these, one died *in utero*. However, virus and antibody was present in all 4 foetuses. Although only 2 out of the 6 foetuses were inoculated in Sow 11, virus was isolated from these and from 2 of the controls, while antibody was detected in both the infected foetuses and one control. Four of the 7 foetuses of Sow 12 were inoculated. Eleven days later virus was isolated from the infected foetuses and one control while all 5 of these and another control foetus had a HI titre. In the third control foetus not enough serum was available for antibody determination (Table 2).

Discussion

Experimental inoculation of gilt foetuses *in utero* at various stages of gestation (27–96 days) with a local strain of PPV resulted in resorption, abortion, mummification and birth of weak or dead piglets. Gentle handling of the gravid uterus in various animal species has no detrimental effect on the foetus(es) and does not result in abortion (Reynolds & Paul, 1955; Jackson, Clarke & Egdahl, 1960; Morgan, Rosenkrantz & Hill, 1966). Furthermore, according to Kramer (1965), intra-uterine subcutaneous and intramuscular injections *per se* do not cause foetal death. It is unlikely that any abortion, resorption or mummification of foetuses in this study can be attributed to the surgical procedures, since none of these effects occurred in the 2 control sows.

According to Mengeling & Cutlip (1976) and Rodeffer, Leman, Dunne, Cropper & Sprecher (1975), early embryonal death resulting in small litter size and mummified foetuses is an important sequel to PPV infection during the early stages of pregnancy, while abortion was not considered important by these authors. Forman *et al.* (1977) described abortions, mummifications and still-born piglets in a piggery where PPV was isolated from foetuses. Johnson & Collings (1969) reported the isolation of PPV from aborted foetuses where the pregnant sows were infected experimentally.

Embryonal death resulting in resorption which was observed by us in sows inoculated between 27 and 71 days of gestation is in accordance with the findings of Mengeling & Cutlip (1975), Mengeling & Cutlip (1976), Rodeffer *et al.* (1975) and Joo & Johnson (1976). Still-birth of piglets infected with PPV was reported by Joo & Johnson (1976) and Forman *et al.* (1977).

From our results it seems that the perivascular inflammatory reactions in foetuses in various organs, especially in the brain and kidney, were dependent upon the age of the foetus at the time of infection. Lesions were pronounced in foetuses inoculated at 71 days, much less severe in foetuses inoculated at 79 and 80 days and absent in foetuses inoculated at 96 days. Older foetuses are thus possibly less susceptible to PPV infection and this could explain the absence of vascular lesions in the latter. Joo & Johnson (1976) stated that the major foetal damage occurs before the onset of immune competence. In foetuses exhibiting a pronounced perivascular inflammatory reaction, this lesion was usually present in various organs. On the other hand, in cases where the perivascular reaction was less severe, it was found mostly in the meninges and pelvic region of the renal medulla, while in more pronounced cases the perivascular infiltrate also extended to blood-vessels in the brain substance. However, in some of the foetuses infected at 79 and 80 days, the perivascular reaction was very mild and only focal infiltrations were occasionally observed. As mild hepatic lesions were seen in only 2 of the foetuses, the significance of these changes is not clear. Mengeling & Cutlip (1975) demonstrated high concentrations of virus antigen in various tissues, including the liver and lungs, with immunofluorescent microscopy.

According to Mengeling, Cutlip, Wilson, Parks & Marshall (1975) and Cutlip & Mengeling (1975) no significant histopathological changes were observed in foetuses or piglets experimentally infected with PPV. In contrast Lenghaus *et al.* (1978) reported necrosis of cells in developing organ systems with mineralization and intranuclear inclusions in the liver, lung, kidney and cerebellum of foetuses inoculated with PPV at 35, 50 and 60 days of gestation. These lesions were not observed in the material examined by us. The vascular lesions seen in the foetuses from sows infected by us at different stages of gestation are in accordance with those recorded by Hogg *et al.* (1977) and subsequent workers. Narita *et al.* (1974) reported the absence of the vascular lesions in the cerebellum, while lesions were found in this locality in our material.

A bronchopneumonia was observed in 4 of the piglets infected at 96 days of gestation. They were born either dead or weak 10 days after infection and died shortly after birth. These piglets had been infected directly into the amniotic fluid. Similar lesions have been described in piglets infected intramuscularly and in piglets examined during a natural outbreak of PPV in a piggery (Hogg *et al.*, 1977; Forman *et al.*, 1977). Therefore, the route of infection does not appear to be a determining factor for the development of lung lesions. Lenghaus *et al.* (1978) reported focal areas of necrosis in the lungs of foetuses infected at 35, 50 and 60 days of gestation, but no overt pneumonia. From these findings it would appear that the extent of the lung lesions observed in foetuses infected with PPV at different stages of gestation is age related, and ranges from focal areas of necrosis to a bronchopneumonia. No other lesions were observed apart from a lymphocytic proliferation around the central arteries in the spleen in piglets with pneumonia. Lesions found in the lymphoreticular system are in accordance with those observed by Hogg *et al.* (1977).

Lesions in the uterine blood-vessels have not previously been reported. From the extent of this change it is deduced that the blood flow to the foetuses may be impaired and therefore contribute to the birth of weak or dead piglets or the termination of pregnancy. However, since in some of the infected foetuses or piglets the vascular reaction was relatively slight or even absent, the direct effect of the virus on proliferating tissue should be considered an important cause of foetal death or the birth of stunted piglets.

Bachmann, Sheffy & Vaughan (1975) reported the intra-uterine spread of PPV in serologically negative sows where the foetuses were infected *in utero*. This was confirmed by us as spread of PPV occurred in 2 sows, 1 of which was serologically negative and one serologically positive at the time the foetuses were infected. Although virus and antibody were present in the control foetus of the serologically positive sow, no histopathological lesions other than a lymphoid proliferation around the central arteries of the spleen was seen.

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