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FETAL INFECTION WITH PORCINE PARVOVIRUS IN HERDS WITH REPRODUCTIVE FAILURE

By

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SØRENSEN, K. and J. ASKAA: *Fetal infection with porcine parvovirus in herds with reproductive failure.* Acta vet. scand. 1981, *22*, 162—170. — During a 20 months period in 1978 and 1979 aborted, macerated and mummified fetuses as well as stillborn piglets from herds with reproductive failure were examined for evidence of infection with porcine parvovirus (PPV). A total of 602 cases were examined and evidence of infection with PPV was found in 269 (45 %). In 52 % of these antibody to PPV was found. Infective PPV as well as antibody to PPV were found in 41 %, whereas infective PPV alone was found in 7 %. When abortions were excluded from the results a high prevalence of infection with PPV (73—90 %) was found among fetuses of all sizes with the exception of fetuses dead late in gestation or at term.

porcine parvovirus; pig fetuses; reproductive failure.

Several reports concerning occurrence of porcine parvovirus (PPV) in dead fetuses have appeared (*Cartwright & Huch* 1967, *Johnson* 1969, 1973, *Morimoto et al.* 1972, *Rondhuis & Straver* 1972, *Mengeling et al.* 1975, *Donaldson-Wood et al.* 1977, *Forman et al.* 1977, *Horner & Hunter* 1977, *Mengeling* 1978). Embryonic and fetal deaths have been induced experimentally by infecting susceptible gilts and sows (*Mengeling & Cutlip* 1976, *Joo et al.* 1976 a, 1977, *Mengeling et al.* 1980), indicating that PPV is a common cause of reproductive failure in pigs. Furthermore, abattoir studies have demonstrated a high prevalence of the infection in fetuses from small litters (*Cropper et al.* 1976) and in fetuses from litters with macerated and mummified fetuses and remnants of embryos (*Mengeling* 1978).

In the present work the prevalence of infection with PPV in dead fetuses from Danish pig breeding herds with reproductive disorders was estimated.

MATERIALS AND METHODS

Specimens

During a period of 20 months in 1978 and 1979 all aborted, macerated and mummified pig fetuses as well as stillborn piglets received for diagnostic laboratory investigation* from herds with cases of fetal death were examined for evidence of infection with PPV.

Samples of lung and liver tissues (usually about 8 g) were collected and pooled under sterile conditions for virus isolation.

Fetal fluids from the thoracic cavity or, in case of complete dehydration, extracts in PBS of brain tissue were collected for antibody examination. Fetuses of similar size from the same herd had their samples pooled. Thus a case may yield one or more samples depending on the sizes of the fetuses submitted.

Cell cultures

Primary cultures of pig kidney cells (PPK) from 2—3-week-old pigs were grown in 11 × 4 cm glass bottles (Schott bottles). Growth medium consisted of modified Hank's balanced salt solution containing 0.75 g $NaHCO_3$/l and supplemented with 5 % fetal calf serum, 0.5 % lactalbumin hydrolysate, a vitamin solution as used in Eagle's MEM, (Glasgow modification), neomycin (0.05 mg/ml) and dihydrostreptomycin (0.1 mg/ml). Maintenance medium was Earle's salt solution supplemented as mentioned above.

Serum

Anti PPV hyperimmune serum was produced in rabbits by 3 subcutaneous inoculations of about 1 µg of sucrose gradient purified PPV (strain 893) (*Lei et al.* 1980) emulsified in Freundt's incomplete adjuvant. The inoculations were performed at weekly intervals. Serum was stored at —20°C until use.

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Detection of virus

Homogenates (10 %) were prepared by grinding the tissues in a Colworth Stomacher 80 (A. J. Seward, Burry st., Edmunds, Suffolk) for about 2 min with serumfree maintenance medium with additional antibiotics, i.e., penicillin (1000 i.u./ml), dihydrostreptomycin (1 mg/ml) and polymyxin B (1000 i.u./ml). The homogenates were clarified by low speed centrifugation for 20 min. From each sample 8 ml were transferred to a Schott bottle containing a 40—50 % confluent monolayer of PPK cells, which had been washed twice in serumfree maintenance medium before use. After adsorption for 1 h at 37°C the cell cultures were drained and washed twice before addition of maintenance medium. After 5 days of incubation at 37°C the cells were subcultured. Volumes of 2 ml containing about 5×10^4 cells/ml in maintenance medium were added to 1×3 cm tubes with coverslips (Leighton tubes) and incubated at 37°C. Control cultures, which were mock-infected with maintenance medium with additional content of antibiotics as described, were maintained in parallel. After 5 days of incubation, cultures from 2 Leighton tubes were examined for evidence of PPV replication by immunofluorescence microscopy (*Sørensen et al.* 1980). Rabbit anti PPV hyperimmune serum (produced as mentioned above) and FITC conjugated anti rabbit IgG serum (DAKO-immunoglobulins Ltd., Copenhagen) served as immuno reagents for identification of PPV.

Detection of antibody

Fetal fluids were tested for specific antibody using the indirect immunofluorescent antibody test (IFAT) and the counter-immunoelectrophoresis test (CIET) (*Sørensen et al.*).

RESULTS

A total of 602 cases were examined for evidence of infection with PPV (Table 1). In 139 cases antibody to PPV in fetal fluids was the only evidence of infection. PPV as well as antibody to PPV were found in 110 cases, whereas PPV alone was found in 20 cases. In total, evidence of infection with PPV was found in 269 (45 %) of the cases examined.

Abortions comprised 132 cases, and of those infection with PPV was found in 10 (Table 2).

Table 1. Demonstration of PPV antibody and infective PPV from 602 cases of fetal death in commercial pig breeding farms.

	Number of cases	%
PPV antibody alone	139	52[1]
PPV antibody and PPV	110	41
PPV alone	20	7
Total PPV positive	269	45[2]

[1] out of 269 positive.
[2] out of 602 cases.

The 602 cases examined comprised 642 samples, where the crown-rump measures were recorded. Table 2 summarizes the prevalence of infection with PPV in the fetuses grouped according to crown-rump measures. Excluding abortions from the data, the prevalence of infection with PPV was very high (73—90 %) among fetuses of all sizes with the exception of fetuses dead late in gestation or at term. Antibody to PPV was readily detected in fetuses of all sizes down to crown-rump measures of about 12 cm. PPV was isolated most frequently from fetuses with crown-rump measures of less than 12 cm and least frequently from fetuses dead near or at term. Clear cytopathic effects of viral replications were occasionally observed, but generally infection of the cell cultures was recognized by immunofluorescence microscopy.

Table 2. Prevalence of infection with PPV according to crown-rump measures of 642 samples of fetuses and groups of fetuses of similar size.

Crown-rump measures (cm)	All		Abortions	Non-abortions	
<10	8/45[1]	(18 %)	0/34[1]	8/11[1]	(73 %)
10—13	47/74	(64 %)	0/22	47/52	(90 %)
14—17	60/86	(70 %)	3/13	57/73	(78 %)
18—21	59/111	(53 %)	4/41	55/70	(79 %)
22—25	36/85	(42 %)	3/22	33/63	(52 %)
26—29	10/29	(34 %)	—	10/29	(34 %)
Stillborn	82/212	(39 %)	—	82/212	(39 %)

[1] PPV positive/total number examined.

DISCUSSION

Losses from reproductive disorders are of great concern in Danish pig breeding herds. *Nielsen & Bendixen* (1969) found that 8.7 % of piglets born by gilts were stillborn or mummified. *Bille et al.* (1974) reported, that 5.9 % of piglets were stillborn or mummified, and in a recent investigation (*Anon.* 1979) mummification was found in 3.8 % of the litters examined.

In the present work evidence of infection with PPV was found in 45 % of the cases examined showing that the infection is commonly found in connection with fetal death in Danish pig breeding herds.

Infection with PPV can also cause embryonic death (*Mengeling* 1979, *Mengeling et al.* 1980, *Wrathall & Mengeling* 1979) resulting in return to estrus or "pseudopregnancy" with persistent corpora lutea without the presence of live embryos (*Rodeffer et al.* 1975), or birth of small litters (*Cropper et al.* 1976). Such cases are not recorded in the present work, since they usually leave no dead fetuses for laboratory investigation.

The material examined comprised 132 cases which were recorded as abortions. PPV was isolated from 10 of these. Also *Cartwright & Huck* (1967) isolated PPV from aborted fetuses. These findings, however, may be coincidences, since abortion due to fetal death in pig is uncommon. In fact, a prolongation of the gestation period tends to take place if all fetuses eventually die (*Wrathall* 1975). This is supported by *Donaldson-Wood et al.* (1977), who found that abortion was not a sequel to PPV infection in a susceptible pig herd, and by *Gillich* (1977), who found no evidence of increase in the number of abortions during an outbreak of PPV induced reproductive failure. Neither have abortions been a sequel to experimental infections with PPV (*Mengeling & Cutlip* 1975, 1976, *Cutlip & Mengeling* 1975, *Joo et al.* 1976a). When abortions were excluded from the present investigation a very high prevalence of infection with PPV was found among fetuses of all sizes with the exception of those dead late in gestation or at term. *Mengeling* (1978) found a similar prevalence of infection with PPV in dead fetuses from slaughtered sows. The results are also in agreement with data from experimental infections with PPV resulting in mummification, stillbirth or birth of apparently normal piglets, with the highest mortality when the infection occurred before the last

part of the mid third of gestation (*Mengeling & Cutlip* 1975, 1976, *Cutlip & Mengeling* 1975, *Joo et al.* 1976a).

The porcine fetus has been found able to produce antibody after stimulation with antigen at about 2 months of gestation (*Binns* 1967, *Fennestad et al.* 1968, *Bourne et al.* 1974). However, after infection of fetuses with subsequent death and mummification it can be difficult to relate exact gestational age to crown-rump measures possibly due to shrinkage of young collagen in intervertebral discs (*Joo et al.* 1976a) and probably also due to growth retardation in a variable period of time from infection to death. Infected fetuses in the present study with crown-rump measures of about 12 cm may therefore have been alive until a gestational age of about 60 days or more and been able to mount an antibody response. *Joo & Johnson* (1977) applied antibody assays for routine diagnosis too, but by using hemagglutination inhibition (HI) tests for demonstration of antibody in fetuses with crown-rump measures > 18 cm. They avoided fetuses with crown-rump measures 16 ± 1 cm in routine diagnosis as these fetuses often had neither HI-antibody nor hemagglutinating (HA)-antigen, whereas smaller fetuses often had high titers of HA-antigen but no HI-antibody. *Joo et al.* (1976 b) also found that fetuses with HI-antibody did not show HA-antigen, although infective virus could be isolated in cell cultures. This was probably due to inhibition of HA-antigen by HI-antibody, and similarly it can be assumed, that excess of HA-antigen in smaller fetuses with crown-rump measures down to about 12 cm prevented the detection of HI-antibody, since antibody in such fetuses could be demonstrated by the methods used in the present study.

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SAMMENDRAG

Infektion med porcint parvovirus i fostre fra besætninger med reproduktionsproblemer.

Over en 20 måneders periode i 1978 og 1979 blev aborterede, macererede og mumificerede fostre samt døde fuldbårne grise fra besætninger med reproduktionsproblemer undersøgt for infektion med porcint parvovirus (PPV). Ialt blev 602 tilfælde undersøgt, og infektion med PPV blev påvist i 269 (45 %). I 52 % af disse fandtes antistof mod PPV. Infektivt PPV samt antistof mod PPV fandtes i 41 %, medens infektivt PPV alene fandtes i 7 %. Når aborterne blev udelukket fra undersøgelsesresultaterne, fandtes infektion med PPV i en stor procentdel (73—90 %) af fostre i alle størrelser med undtagelse af fostre døde sent i drægtighedsperioden eller ved fødslen.

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