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From the State Veterinary Institute for Virus Research,
Lindholm, Kalvehave, Denmark.

ASSAY FOR ANTIBODY IN PIG FETUSES INFECTED WITH PORCINE PARVOVIRUS

By

K. J. Sørensen, J. Askaa & K. Dalsgaard

SØRENSEN, K. J., J. ASKAA and K. DALSGAARD: *Assay for antibody in pig fetuses infected with porcine parvovirus.* Acta vet. scand. 1980, *21*, 312—317. — Fetal fluids from field cases of fetal death were assayed for antibody to porcine parvovirus (PPV) using 3 different techniques. An indirect immunofluorescent antibody test, a counter immunoelectrophoresis test and a hemagglutination inhibition test were compared. The indirect immunofluorescent antibody test was found to be the most sensitive of the tests employed. The hemagglutination inhibition test apparently suffered from the occurrence of false positive results.

indirect immunofluorescent antibody test; counter immunoelectrophoresis test; hemagglutination inhibition test; porcine parvovirus; porcine fetus.

The porcine fetus is capable of mounting an antibody response, when stimulated with antigen after about 2 months of gestation. Thus porcine parvovirus (PPV) has been found to pass the porcine placenta and to elicit an antibody response in the fetus (*Johnson & Collings* 1971). In order to detect the antibody response sensitive methods are important. So far the hemagglutination inhibition test (HIT) (*Bachmann* 1969, *Cartwright et al.* 1969, *Mengeling* 1972, *Johnson* 1973, *Joo et al.* 1975) has been used for assay of antibody to PPV. Also serum neutralization tests have been applied (*Mengeling* 1972, *Joo et al.* 1975). In the present work an indirect immunofluorescent antibody test (IFAT), a counter immunoelectrophoresis test (CIET) and the HIT were compared. Fetal fluids from field cases of fetal death were assayed for antibody to PPV using the 3 different techniques.

MATERIAL AND METHODS

Clinical specimens

Fluids from the thoracic cavity of fetuses from herds with cases of fetal death were examined. In case of complete dehydration extracts in PBS of brain tissue were used. When several fetuses from the same herd were received for examination samples from fetuses of about the same size were pooled. A total of 133 samples were examined.

Virus

PPV isolated from field material was grown in primary pig kidney cells (PPK cells) nourished with medium consisting of Earle's salt solution with lactalbumin hydrolysate (0.5 %), a vitamin solution as used in Eagle's MEM, bovine fetal serum (7 %), neomycin (100 U/ml) and streptomycin (0.1 mg/ml). A cell suspension with 10^5 cells per ml was infected at a multiplicity of about 1.0 and seeded in 1 l roller bottles. After occurrence of cytopathic effect the cells were scraped off the glass surface and packed by low speed centrifugation for 10 min. The pellets were resuspended in 0.2 M glycine buffer pH 9.0 using 5 ml per bottle (*Hallauer et al.* 1971). After stirring for 30 min at room temperature and high speed centrifugation for 10 min the supernatant was stored at —80°C until use.

Indirect immunofluorescent antibody test (IFAT)

A PPK cell suspension containing 4×10^5 cells per ml was infected at a multiplicity of about 0.5 and seeded in leighton tubes with coverslips. After incubation for about 20 h the coverslips with infected monolayers were removed, fixed in acetone for 10 min and stored at —20°C until use. Diluted samples were incubated on the coverslips for 60 min at 37°C. After 4 washings of 5 min each in PBS the coverslips were incubated with diluted FITC conjugated rabbit anti swine serum IgG (immunization procedure as described by *Harboe & Ingild* 1973, conjugation procedure as described by *The & Feltkamp* 1970) for 30 min at 37°C. After another 4 washings (3×5 min in PBS and 1×5 min in distilled water) the coverslips were mounted in glycerine-elvanol and read in a Leitz Orthoplan fluorescent microscope. Titers were expressed as the reciprocal value of the highest dilution giving visible fluorescence. As controls served diluted PPV antibody negative and PPV antibody positive sera.

Counter immunoelectrophoresis test (CIET)

The CIET was performed on 10×10 cm glass plates with 1.5 mm gels of 1 % agarose with relatively high endosmosis (Type LSA, Litex, Glostrup, Denmark). The buffer consisted of barbitone sodium 5 g, barbitone 1 g, sodium azide 0.2 g and distilled water to make 1 l, pH 8.6. Pairs of wells 3 mm in diameter and 10 mm apart (centre to centre) were punched in the gel. Undiluted or serial diluted fetal fluids were applied to the anodic wells and the PPV antigen in optimal dilution to the cathodic wells both in 15 μl volumes. Electrophoresis

was performed overnight at 1.5 v/cm, and the plates were pressed, dried and stained with Coomassie Brilliant Blue®. Titers were expressed as the reciprocal value of the highest dilution giving a detectable precipitin line.

Hemagglutination inhibition test (HIT)

The HIT was performed as described by *Joo et al.* (1975).

Rocket immunoelectrophoresis

The technique described by *Dalsgaard et al.* (1979) was used.

RESULTS

When testing PPV antibody positive samples of fetal fluids using the IFAT, distinct nuclear fluorescence was seen in infected cells on a dark background of non-infected non-fluorescent cells. The CIET gave distinct precipitin lines with PPV antibody positive samples. From Table 1 it is seen that with 51 samples all 3 tests were positive, with 12 samples only the IFAT and either the CIET or the HIT were positive, and with 5 samples antibody could be detected by the IFAT only. In 4 of the latter 5 cases additional evidence of infection with PPV was found by the isolation of PPV from organs of the respective fetuses in 3 cases, 1 of which also had increased Ig levels, or by demonstration of antibody in litter mates of different body size by all 3 tests (1 case). Of the remaining 65 samples 37 reacted in the HIT only. Thirty had titers between 8 and 32, and 7 had titers between

Table 1. Antibody to PPV in fluids from pig fetuses grouped according to reaction pattern.

Number of samples	IFAT[1]	CIET[1]	HIT[1]	Increased Ig[2]
51	51/51	51/51	51/51	43/51
12	12/12	7/12	5/12	7/12
5	5/5	0/5	0/5	1/5
65	0/65	0/65	37/65	0/65

The samples were used diluted 1/20 in the IFAT, undiluted in the CIET and titrated in the HIT with lowest dilution step 1/8. The values represent 1 determination.

[1] Number of positive reactants/number of samples examined by the IFAT, the CIET and the HIT.

[2] Number of samples with increased content of IgG and/or of IgM/ number of samples examined by rocket immunoelectrophoresis.

64 and 256. No further evidence of infection with PPV was found in these cases, i.e. antibody was not detected by the IFAT and the CIET, virus was not isolated and Ig levels were not increased. Litter mates of different size were not available. On this background the reactions were supposed to be non-specific. In total a significant content of PPV antibody was found in 68 of the 133 samples. The IFAT was positive in all 68, whereas 58 and 56 were positive using the CIET and the HIT, respectively.

The Ig levels of the 133 samples as measured by rocket immunoelectrophoresis are also tabulated (Table 1). It appears that the frequency of PPV antibody positive samples with increased Ig levels was highest, when all 3 tests were positive, and lowest, when only 1 test was positive.

In order to determine whether the better ability of the IFAT to detect antibody to PPV could be attributed to greater sensitivity of the test, 14 samples with PPV antibody were subjected to quantification by the 3 tests (Table 2). It appears that the IFAT consistently gave higher titers than did the CIET and the HIT. The difference between the values obtained by the IFAT and the CIET, and between the values obtained by the IFAT and the HIT, respectively, are significant ($P < 0.005$ in both cases, t-test, *Croxton* 1959).

Table 2. Titer values obtained by the IFAT, the CIET and the HIT.

Sample no.	IFAT titer	CIET titer	HIT titer
1	1280	24	384
2	480	2	24
3	2560	32	256
4	240	3	16
5	640	2	16
6	800	2	32
7	960	1	24
8	960	4	8
10	640	2	8
12	120	1	neg.
9	240	2	8
11	40	1	neg.
13	320	neg.	neg.
14	480	2	neg.

neg. in the CIET means titers < 1 and in the HIT < 8
The values represent the means of 2 determinations.

DISCUSSION

For rapid demonstration of in utero infection with PPV either detection of viral antigen in fetal tissues by hemagglutination tests (*Joo et al.* 1976) and by direct immunofluorescent microscopy on cryostat-microtome sections (*Mengeling* 1978), or demonstration of specific antibody in fetal fluids by hemagglutination inhibition tests (*Joo et al.* 1976) have been recommended. As antibody content in fetuses infected with PPV may be low, sensitive methods are desirable. In this work the IFAT was found to be the most sensitive of the tests employed. When preparing the infected monolayers as described, fluorescent antigen was almost restricted to the nucleus of infected cells, which appeared with very distinct fluorescence in strong contrast to the surrounding non-infected non-fluorescent cells. The absence of a non-specific background fluorescence, which often occurs when sera from adults are tested, can probably be ascribed to the low protein content of fetal fluids compared to serum from adults. As the IFAT in addition was found to be convenient and rapid with relatively few efforts required for the preparation of antigen, it was preferred for PPV antibody assay in fetal fluids. The HIT and the CIET were found less sensitive, and in addition the HIT apparently suffered from the occurrence of false positive results.

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SAMMENDRAG

Undersøgelser for antistof i grisefostre inficeret med porcint parvovirus.

Fostervæsker fra besætninger med formodet forekomst af smitsom fosterdød er undersøgt for indhold af antistof mod PPV ved 3 forskellige teknikker. En indirekte immunofluorescens antistoftest, en counter immunoelectroforesetest og en hæmagglutinationsinhibitionstest er sammenlignet. Den indirekte immunofluorescenstest fandtes at være den mest følsomme af de anvendte teknikker. Hæmagglutinationsinhibitionstesten gav tilsyneladende falske positive resultater.

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Reprints may be requested from: K. J. Sørensen, the State Veterinary Institute for Virus Research, Lindholm, DK-4771 Kalvehave, Denmark.