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RAPID METHOD FOR SCREENING OF IMMUNOGLOBULINS IN PORCINE FETUSES, USING ROCKET IMMUNOELECTROPHORESIS

APPLICATION OF AN INTERSPECIES REACTION BETWEEN HUMAN AND PORCINE μ-CHAIN*

By

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DALSGAARD, K., E. OVERBY, J. J. METZGER and A. BASSE: *Rapid method for screening of immunoglobulins in porcine fetuses, using rocket immunoelectrophoresis. Application of an interspecies reaction between human and porcine μ-chain.* Acta vet. scand. 1979, *20*, 313—320. — A method for screening of immunoglobulin levels in porcine fetuses is presented. The method is based on rocket electrophoresis. This method is sensitive, but when used for assaying immunoglobulins it is only semiquantitative. It was shown that sera from 11 hysterectomy-derived colostrum-deprived neonatal pigs from a normal gilt contained no IgM or IgA, but small amounts of IgG. Sera from 9 piglets isolated in the same way from a gilt infected with porcine parvovirus contained all 3 antibody classes. IgM was detected by an interspecies reaction using anti human μ-chain immunoglobulin.

pig fetus; parvovirus; immunoglobulins; rocket electrophoresis.

In the normal porcine fetus only traces of immunoglobulins have been demonstrated. This has been shown to be a 4 S molecule of IgG specificity (*Bourne* 1974). In addition to IgG also IgA has been shown in the serum of precolostral newborn piglets (*Prokesova et al.* 1969, *Jönsson* 1973). After intrauterine stimulation with antigen the porcine fetus has been shown to be immunocompetent after the 55th day of gestation (*Sterzl* 1965, *Binns*

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1967, *Fenneslad* 1968, *Bourne et al.* 1974), the resulting antibody being mainly of the lgM type (*Bourne et al.*).

In the diagnostic laboratory, stillborn and/or mummified porcine fetuses are received from herds with a history of reproductive failure for the diagnosis of a possible causative infectious agent. The method described in this paper was developed for a preliminary screening of such fetuses for the presence of IgM and IgG in pleura- or brain cavity fluid. This could be of value in the detection of a transplacental infection.

MATERIALS AND METHODS

Experimental animals

Two gilts of the Danish landrace were used. The gilts were free from serum antibodies against porcine parvovirus when tested by hemagglutination inhibition (*Joo et al.* 1975). They were artificially inseminated. Pregnancy was shown by the detection of heart sound (Med-Sonics, Ca 94042 USA). On days 42 and 43, 60 ml of tissue culture fluid containing 10^6 TCID50/ml of parvovirus (Danish field strain, Fakse 1976) was injected intraveneously into 1 gilt. The other gilt was isolated and served as a non-infected control. At term hysterectomy-derived colostrum-deprived piglets were isolated. The infected gilt gave 9 fullborn piglets and 2 mummified fetuses (7 and 9 cm). The control gave 11 piglets. Blood samples were taken from all piglets and the serum was isolated after clotting. Serum antibody against parvovirus was estimated by hemagglutination inhibition (*Joo et al.*).

Rocket electrophoresis

Essentially the method of *Weeke* (1973) was followed. 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. Agarose type HSB (Litex, Glostrup, Denmark) was used in a concentration of 1 %, and a layer thickness of 1.5 mm. Five µl undiluted samples were applied to wells 2 mm in diameter punched by a special gel-puncher and template (Dansk Laboratorieudstyr, Copenhagen). The antisera (0.5 ml) were added to the liquid agarose (14.5 ml) at 56°C. Plates 10×10 cm were used. Electrophoresis was carried out overnight at 1.5 V/cm. The plates were pressed, dried and stained with Coomassie Brilliant Blue®, as described by *Weeke* (1973).

Immunochemicals

A reagent specific for porcine γ-chains was prepared in rabbits according to the method of *Metzger & Houdayer* (1972), using resolubilization with 8 molar urea. IgG was isolated as described by *Metzger & Fougereau* (1967). The immunoglobulin fraction (*Weeke* 1973) of rabbit sera was used.

A reagent specific for porcine α-chains was prepared according to the same method, IgA being isolated as described by *Bourne* (1969) and *Curtis & Bourne* (1971).

A reagent specific for human μ-chains was purchased from DAKO Immunoglobulins, Copenhagen, Denmark (Code no. 10-091, lot 037).

RESULTS

The immunoglobulin levels as detected by rocket electrophoresis are shown in Fig. 1. It can be seen that the only class detected in normal piglets was of IgG specificity (Plates 517, 519, 521). In piglets from a gilt infected with parvovirus, however, all 3 antibody classes could be easily detected by this method (Plates 535, 518, 530). An interesting observation was that a commercial antiserum against human μ-chain reacted with porcine serum expected to contain IgM. The results confirmed that

Table 1. Level of hemagglutination inhibiting antibodies against porcine parvovirus in sera from hysterectomy-derived colostrum-deprived neonatal pigs. Titres were estimated in microplates (*Joo et al.* 1975).

Control gilt		Parvovirus infected gilt	
piglet No.	titre	piglet No.	titre
1	< 32	1	> 1024
2	„	2	> 1024
3	„	3	> 1024
4	„	4	512
5	„	5	256
6	„	6	> 1024
7	„	7	> 1024
8	„	8	> 1024
9	„	9	> 1024
10	„		
11	„		

the fetus was able to elicit an immune response. That parvovirus has passed the placental barrier was further confirmed by the demonstration of hemagglutination inhibiting antibodies. The result is shown in Table 1.

DISCUSSION

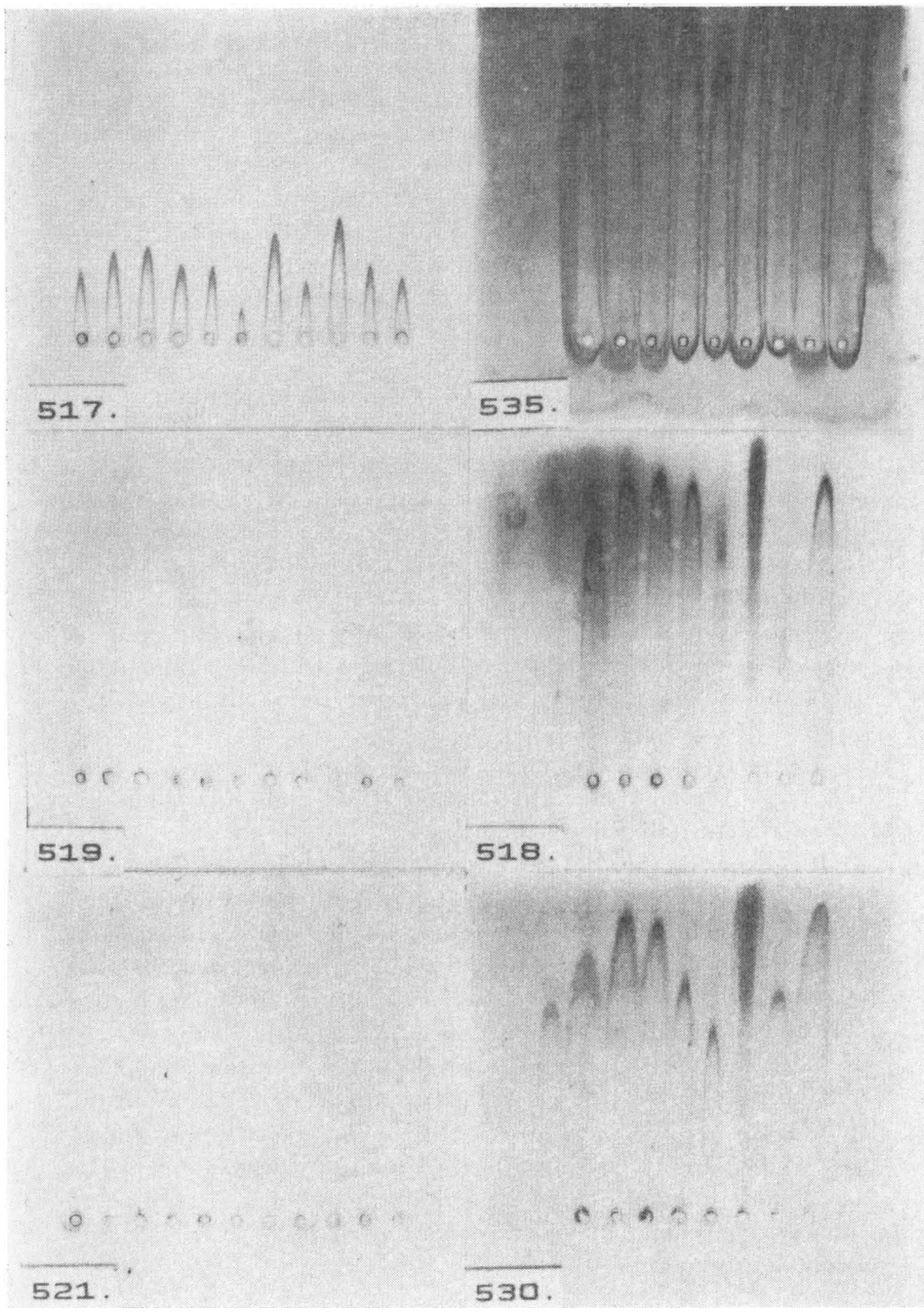
Using rocket immunoelectrophoresis results were obtained which showed that normal hysterectomy-derived colostrum- deprived neonatal pigs do not contain immunoglobulins of the IgM and the IgA class. This is in agreement with the report of *Bourne et al.* (1974) and of *Travnicek & Mandel* (1971), but differs from the results of *Prokesova et al.* (1969) and *Jönsson* (1973). It was demonstrated, however, that immunoglobulins of IgG specificity are present in normal piglets at term, although at relatively low levels. *Bourne et al.* found only trace amounts in some piglets whereas all normal piglets in this report were positive when using rocket electrophoresis. The discrepancies found in the literature may be explained by differences in sensitivity of the detection methods.

After experimental infection with a virus known to cross the placental barrier (*Bourne et al.*), it was clearly shown that the fetus can be immunocompetent and that immunoglobulins of IgG, IgM and IgA classes can be detected. This result is also compatible with the findings of *Bourne et al.* and *Sterzl* (1965). The IgM and the IgA response of the antigenically stimulated fetus was shown to be an all or none reaction. The use of anti human μ-chain or anti IgM in the IgM reaction seems attractive for diagnostic purposes because of the specificity of the reagent. There is no cross reactivity of the porcine L-chain with any other mammalian L-chain (*Metzger & Houdayer* 1972). The cross-reaction between human and porcine μ-chain was reported in 1976

Figure 1. Rocket electrophoresis of serum samples from hysterectomy-derived colostrum-deprived neonatal pigs.

Plates 517, 519, 521: Piglets Nos. 1—11 from a non-infected control gilt, anti porcine γ-chains, anti human μ-chains and anti porcine α-chains, respectively.

Plates 535, 518, 530: Piglets Nos. 1—9 from a porcine parvovirus infected gilt, anti porcine γ-chains, anti human μ-chains and anti porcine α-chains, respectively.


517.
535.
519.
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by *Metzger & Bazin*. This cross-reaction did not exist with bovine IgM.

The electrophoresis system described here included an agarose with low electroendosmosis. This is important for the shape of the rocket, and the system has been designed only with the aim of making it easy to find the positive samples.

The system can not be recommended for quantitation of immunoglobulins. For this purpose, single radial immunodiffusion (*Mancini et al.* 1965) is the method of choice. The latter method, however, suffers from the relatively long time (about 1 week) required for the diffusion, because of the high molecular weight of IgM.

Several factors play a role in determining whether the fetus will elicit an immune response. 1: The fetus is not immunocompetent until the 55th day of gestation. 2: The incubation period of the virus infection may play a role. 3: The incubation period and the persistence of the virus in utero may play a role. 4: Viruses producing phases of cyclic viremia in a sow with a possibility of generalization to the fetus may play a role.

Binns (1967) using bone marrow and lymph node cells as antigen found a high degree of tolerance with injection at 60 days, but cellular immune reactions occurred when antigen was given at 80 days gestation.

The plates containing anti μ-chain reagent are easy to evaluate: rocket or no rocket. Estimation of the anti IgG plates involves a quantitative judgement.

It is expected that the detection of IgM and/or increased levels of IgG in the porcine fetus can be taken as a sign of intrauterine infection. No information is obtained about the infectious agent whether it is bacteria or virus etc., but it is suggested that the screening for IgM and IgG in stillborn and/or mummified piglets may be valuable in supporting a specific diagnosis. In the present investigation electrophoresis overnight at low voltage was applied. But the reaction may be performed in 3 h at 10 V/cm. With the staining procedure used (*Weeke* 1973) the results may consequently be obtained within 5 h after receipt of the samples. The use of anti human μ-chain reagent for porcine IgM may be of value for veterinary diagnostic laboratories, because it is readily available commercially.

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SAMMENDRAG

Raket immunelektroforese som en hurtig metode til "screening" af immunglobuliner i grisefostre. Anvendelse af en interspecies reaktion mellem human og porcin μ-kæde.

En metode til "screening" af immunglobulinniveau i grisefostre fremlægges. Metoden er baseret på raketelektroforese. Denne metode er følsom, men kun semikvantitativ ved bestemmelse af immunglobuliner. Det påvistes, at sera fra 11 grise, som blev taget ved hysterektomi, og som ikke fik kolostrum, indeholdt små mængder IgG, men ikke IgM eller IgA. Sera fra 9 tilsvarende grise, som blev taget fra en gylt der var inficeret med porcint parvovirus, indeholdt alle 3 antistofklasser. IgM blev påvist ved en interspecies reaktion under anvendelse af anti human μ-kæde immunglobulin.

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