IMMUNOGLOBULINS IN BLOOD SERUM OF FOETAL PIGS

T. D. CHANIAGO*, D. L. WATSON*, R. A. OWENT† and R. H. JOHNSON*

SUMMARY: A total of 1,147 samples of blood serum, collected from porcine foetuses, were examined for the presence of immunoglobulin. The foetuses, from 182 sows, were sampled at abattoirs in Queensland during 1975. For detection and measurement of immunoglobulins, rabbit anti-pig serum and monospecific anti-pig IgG, anti-pig IgM and anti-pig IgA were employed in immunoelectrophoresis, double diffusion and single radial immunodiffusion assays. Twenty-four foetuses (from 7 litters) had detectable IgG or IgM. None of the samples were positive for IgA. Two of the sera (from siblings) had high antibody titres to porcine parovirus but in the remainder of the immunoglobulin-positive sera no antibody activity was detected.

Introduction
There is normally no transmission of immunoglobulin across the placenta of the pig and in consequence piglets are normally born agamaglobulinic (Sterzl et al 1965; Porter 1969b). There are two possible causes, however, for the presence of immunoglobulin in the circulation of foetal pigs. Firstly, if there is damage to the placenta then maternal immunoglobulin, together with other serum proteins, may reach the foetal circulation (Kim et al 1966; Wellmann et al 1972). Secondly, it is known that the foetal pig becomes immunocompetent at about 70 days gestation (Binns 1973), and there is ample evidence that antigens introduced to the foetus after this stage may stimulate an antibody response by the foetus (Binns 1969; Hajek et al 1969; Tlaskalova et al 1970).

The aim of the experiments described in this paper was to examine, using sensitive immunological techniques, a large number of serum samples from apparently normal foetal pigs and thereby determine the incidence and age distribution of foetuses with circulating immunoglobulin. An attempt was made to identify the antigens against which the immunoglobulin had antibody activity.

Materials and Methods
Collection and Preparation of Samples
Samples of foetal pig blood were collected from abattoirs in Queensland during 1975. All blood samples were collected by cardiac puncture of the foetus as soon as possible after death of the sow. Serum was prepared from blood within 12 hours of collection and stored at —16°C until required for analysis.

At the time of slaughter the following information was recorded: crown to rump length of each foetus, number of foetuses per litter and whether the dam was primiparous or multiparous. The volume of blood obtained from some foetuses was too small to be assayed and in these cases sera from individual foetuses within the litter were pooled.

Calculation of Foetal Age
The gestational age of each foetus was estimated according to the following formula (Marrable 1971):

\[ T = (L + 70.59) - 3.25 \]

where T is gestational age (days)

L is crown to rump length (mm)

Preparation of Immunoglobulins and Antisera
Pig IgM, IgG and IgA were isolated as described by Porter (1969a). Antisera to the purified immunoglobulin preparations were raised in rabbits (Brandon et al 1971) and rendered monospecific by affinity chromatography. For this purpose glass columns (10 mm x 180 mm) were packed with cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala) coupled to purified porcine IgG or IgM. Protein coupling, blocking of active groups and elution of antisera were carried out according to the manufacturer’s recommendations.

Monospecificity of each antiserum was routinely checked by immunoelectrophoresis and double diffusion in agar, against purified IgG, IgM and IgA as well as porcine colostral whey and pooled serum.

Rabbit anti-pig serum with strong reactivity against the albumins, α-, β- and γ-globulins was used in immunoelectrophoresis assays.

Immunoelectrophoresis
Immunoelectrophoresis was carried out for exactly 1 hour (300 volts, 10 milliamperes per 200 mm) according to the micromethod of Scheidegger (1953). On each immunoelectrophoretic plate there were 10 samples of foetal pig serum and 2 pooled adult pig sera as positive controls. Rabbit anti-pig serum was used to develop precipitin lines.

Double Diffusion in Agar
A modification of the method of Ouchterlony (1958) was used for double diffusion tests. Wells (2 mm diameter) were punched in slabs of 1% agar on microscope slides. Six holes were punched around the circumference of a circle (diameter 20 mm) with a central well being punched for antisera. Exactly 5 μl of antisera or foetal pig serum was placed in each well and the plates were then kept in a humid chamber for 36 to 48 hours.

Single Radial Immunodiffusion
The concentration of immunoglobulins in serum samples


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was determined using a modified single radial immunodiffusion method (Brandon et al 1971).

**Antibody Assays**

Antibody to porcine parvovirus was assayed using a haemagglutination inhibition (HI) technique (Joo et al 1975).

An indirect haemagglutination test (Buxton and Tomlinson 1961) was employed to detect antibody to *Escherichia coli*. Sheep erythrocytes were modified with lipopolysaccharides prepared from *E. coli* 0141.

The Rose-Bengal plate test was used to detect antibody to *Brucella suis* (Morgan et al 1969).

An agglutination-lysis test was used to detect antibody to *Leptospira hardjo*, *L. tarassovi* and *L. pomona* (Winks 1962).

**Experimental Procedure**

Each sample of foetal pig serum was subjected to analysis by immunoelectrophoresis (using polyvalent antiserum) and double diffusion (using monospecific antisera). Any samples which were positive for immunoglobulin in either of these assays, were then tested by single radial immunodiffusion and subjected to each of the antibody assays.

**Results**

A total of 1,147 samples of foetal pig serum was examined. These samples were derived from 1,221 foetuses, the discrepancy being due to pooling of very small samples from foetuses within some litters. The foetuses belonged to 182 litters with the mean number of foetuses per litter being 6.71 (range 1 to 14). There were 68 primiparous and 114 multiparous sows involved in the study.

Calculated gestational ages of foetuses ranged from 46 to 106 days with a mean of 74 days. The number of foetuses in various age groups is shown in Figure 1.

Results for those sera which were immunoglobulin positive are shown in Table 1. A total of 24 foetal serums from 7 litters contained detectable levels of immunoglobulin. Of these, 3 had only IgM, 19 only IgG and 2 contained both IgM and IgG. IgA was not detected in any sample. Of the serums which were positive for IgG on immunoelectrophoresis or double diffusion, only 4 had quantities sufficient to be measured by single radial immunodiffusion. The minimum measurable concentration of porcine IgG by this technique is approximately 0.05 mg/ml.

Each of the 24 immunoglobulin-positive serums were tested for antibody to porcine parvovirus, *E. coli*, *Brucella sp.* and *Leptospira spp*. All serums were negative for antibody to *E. coli*, *Brucella sp.* and *Leptospira spp*. Two samples (K1-3 and K1-5) were positive for HI antibody to porcine par-

### TABLE 1

<table>
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<tr>
<th>Litter No.</th>
<th>Foetal Age (Days)</th>
<th>No. of Foetuses</th>
<th>Ig positive Poetal Sera</th>
<th>IgM (mg/ml)</th>
<th>IgG (mg/ml)</th>
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<td></td>
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<td>5</td>
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*Australian Veterinary Journal, Vol. 54, January, 1978*
vovirus, each having a titre of 800; the remainder of the sera were negative in this assay.

Discussion

Since the pioneering studies of Young and Underdahl (1949, 1950) it has been widely accepted that there is normally no transmission of maternal immunoglobulin across the epitheliochorial placenta of the pig (Brambell 1970). The results obtained from the present survey confirmed this tenet, but at the same time revealed a small percentage of foetuses which contained maternally-derived or autologous immunoglobulin. All foetuses which had circulating immunoglobulin were beyond the age of onset of immunocompetence, thus the possibility existed for the immunoglobulin, in each case, to have resulted from autologous production or leakage across a damaged placenta. The latter possibility is most unlikely to pertain to foetuses D118-4 or D37-3 since in these cases there were measurable quantities of IgM yet no detectable IgG. It is inconceivable that IgM could leak through placental barriers without concomitant leakage of IgG, bearing in mind the molecular size of each immunoglobulin class and their respective concentrations in sow serum (Porter 1969b). Clearly, the immunoglobulin present in serum from these 2 foetuses represented autologous antibody responses by the foetuses (presumably primary responses) to one or more unknown antigens. Foetuses K1-3 and K1-5 which had high antibody titres against parvovirus, as well as circulating immunoglobulin, also appeared to be good examples of the foetal immune response (Bourne et al 1974).

For serum from foetus K29-3 the concentration ratio for IgG : IgM was 1.6 : 1 which was greatly different from values for adult sow serum of 20 : 1 (Porter, 1969b) and 7 : 1 (D. L. Watson, unpublished data). This evidence strongly suggests that the immunoglobulin in serum from K29-3 was produced by the foetus.

The situation regarding the origin of immunoglobulin in the remaining foetuses (from sows K10, K46 and D1) is less predictable. The IgG present in these cases may reflect an autologous immune response in the foetus. However, in view of the absence of any detectable IgM and the extremely low levels of IgG involved, it seems more likely that damage to, or minor imperfections in, the placental barriers explain these results.

The relatively small quantities of serum obtained from each foetus prohibited the testing of the immunoglobulin-positive sera against a wider range of potential antigens. Given sufficient serum there would be a good case for testing for antibody to pathogens such as Listeria monocytogenes, Staphylococcus spp., Streptococcus spp., Corynebacterium spp. (Blood and Henderson 1974), other serotypes of Leptospira, SMED1 virus (Wang et al 1973) and blood or tissue cells of the dam. Because of the very high levels of antibody to E. coli in serum of adult sows (Watson 1975), it was considered that detection of antibody of this specificity in foetal serum would be a strong indication that it was maternally-derived.

Acknowledgments

We thank staff of the Animal Health Station, Queensland Department of Primary Industries for carrying out antibody assays to Leptospira spp. and Brucella suis. The skilled technical assistance of Mrs M. A. Bennell is gratefully acknowledged. This work was supported by grants from the Australian Pig Industry Research Committee.

References


(Received for publication 15 March 1977)