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Serum Antibodies and Immunoglobulins of Newborn Calves

By

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With 6 figures and one table

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Unlike the human foetus which is endowed with immunoglobulins already in utero, the young of some domestic animals, particularly piglets and calves, are born without satisfactory amounts of immunoglobulins in consequence of a different structure of the placenta. Small quantities of gamma globulins in piglet precolostral serum have been demonstrated by a number of investigators (Franěk et al., 1961; Segre and Kaebler, 1962; Wellmann, 1963) and, more recently, evidence has been obtained to indicate an antibody character of this immunoglobulin which is similar to IgG of adult pigs in its antigenic properties but differs from it by its molecular weight (Myers and Segre, 1963; Franěk and Řihá, 1964; Menšík and Franz, 1968; Prokešová et al., 1969; Franz et al., 1971). Calf precolostral serum was repeatedly reported (Smith and Holm, 1948; Jones, 1967; Rice and Carriere, 1969; Stone, 1969, 1970) to contain no immunoglobulins, even though other writers (Kniazeff et al., 1967; McCoy et al., 1967; Menšík et al., 1970; Merriman, 1971) did demonstrate immunoglobulin in precolostral calf serum and some of them were even able to characterize it in part.

The present study is concerned with immunoglobulins in precolostral sera of calves from dams immunized with parainfluenza 3 (PI-3) virus and Mycoplasma bovirhinis.

Material and Methods

Experimental Animals

Three groups of experimental calves were employed. Group 1 consisted of 6 calves born from dams vaccinated with two subcutaneous doses of live PI-3 virus and live Al(OH)₃-adsorbed M. bovirhinis vaccine 20 and 40 days before parturition, with both vaccines being administered at the same time. Group 2 consisted of 6 calves born from dams immunized with a single dose of live PI-3 virus 10 to 30 days before parturition. Group 3 comprised 3 control calves from unvaccinated dams.

All the cows included in this experiment showed low or medium antibody levels to both antigens employed, even before vaccination.
**Antigens**

The live PI-3 virus employed for active immunization was the T-6 strain originally isolated from the lung of a diseased calf and passaged for a long term in primary calf kidney cell cultures. The titre of infective tissue culture fluid, employed for vaccination in doses of 30 and 20 ml., was $10^4$—$10^7$ TCID$_{50}$/0.1 ml. *M. bovirhinis* antigen was propagated in liquid medium and centrifuged at 4000 r. p. m. for 30 minutes. The sediment was resuspended in calf serum to make 100 times the original volume. Two parts of this antigen were mixed with one part of sterile Al(OH)$_3$ in gel. The adsorbed vaccine thus prepared was stored at $4\, ^\circ\text{C}$ for no more than 7 days before being used for vaccination in a dose of 5—10 ml.

**Serological Examination**

Blood samples were obtained from the jugular vein of neonatal calves and centrifuged at 3000 r.p.m. for 20 minutes. Demonstration of antibodies to PI-3 virus was made by the haemagglutination-inhibition (HI) test. The blood sera were inactivated at 56 $^\circ$C for 30 min. and treated with a 25 per cent suspension of kaolin. Serial twofold dilutions of the sera were mixed in 0.1 ml. amounts with equal volumes of diluted infective tissue culture fluid containing 4 haemagglutinating (HA) units of virus. After incubation for 1 hour at laboratory temperature, 0.2 ml. amounts of a 0.5 per cent suspension of guinea pig erythrocytes were added and the results were read after allowing 1—2 hours for settling at laboratory temperature.

Demonstration of antibodies to *M. bovirhinis* was also made by the HI test. The sera were inactivated at 56 $^\circ$C for 30 min. Serial twofold dilutions of the sera were mixed in 0.1 ml. amounts with equal volumes of antigen containing 8 HA units. After incubation for 1 hour at laboratory temperature, 0.2 ml. amounts of a 0.5 per cent suspension of bovine erythrocytes were added and the mixture was incubated for an additional 1.5—2 hours at laboratory temperature before reading the test.

In both cases the antibody titres were given as the reciprocal of the highest serum dilution inhibiting haemagglutination.

**Preparation of Immunoabsorbent and Isolation of Precolostral IgG**

The immunoabsorbent was prepared by coupling antibodies against bovine IgG to Sepharose 2B (Farmacia Uppsala) activated with cyanogen bromide according to Porath et al. (1967) and Matheka and Mussgay (1969). The specificity of the immunoabsorbent was checked by coupling IgG from adult bovine serum, eluting it and determining the purity of the eluate by means of immunoelectrophoresis.

Eight hundred ml. of pooled serum of neonatal calves from vaccinated cows was precipitated with 33 per cent saturated ammonium sulphate. The precipitate was centrifuged, dissolved in distilled water and dialysed against borate buffer of pH 8.0. From this protein solution, IgG was adsorbed to immunoabsorbent and eluted successively with two glycine buffers of pH 3.4 and 2.4. The pH of the fractions was adjusted to 7.0 and the fractions were concentrated by ultrafiltration and subjected to immunoelectrophoresis and serological examination.

**Gel Filtration**

Blood sera from calves were separated on 2.5 cm. $\times$ 70.0 cm. columns of Sephadex G-200 (Farmacia Uppsala) according to Flodin and Killander.
(1962) and eluted with 0.1 M Tris-HCl buffer (pH 8.0) in 0.2 M NaCl. The eluate was collected and measured spectrophotometrically at 280 nm. The values were presented graphically. The individual fractions were subjected to serological examination and the IgG concentration was determined by quantitative radial immunodiffusion.

**Chromatography on DEAE Cellulose**

Five hundred ml. of pooled serum of neonatal calves from vaccinated cows was precipitated with 33 per cent saturated ammonium sulphate and dialysed against 0.005 M phosphate buffer, pH 8.0 (Sober et al., 1956). The chromatography was performed on 2.5 cm. x 35.0 cm. DEAE cellulose (Serva DEAE SS) columns equilibrated with the same buffer. The proteins were eluted with linear gradient phosphate buffer (0.005—0.3 M, pH 8.0). The eluate was measured spectrophotometrically at 280 nm. and the values were presented graphically. The IgG content of the fractions was determined by quantitative radial immunodiffusion.

**Immunoelectrophoresis**

Immunoelectrophoresis was performed on 9.0 cm. x 15.0 cm. glass plates with 1.5 per cent agar in 0.05 M barbiturate buffer at pH 8.0 using a current intensity of 35 mA. The precipitin lines were labelled with a 0.5 per cent solution of amidoblack 10 B.

**Quantitative Radial Immunodiffusion**

The procedure employed was that described by Fahey (1956) using 9.0 cm. x 15.0 cm. glass plates with 25 ml. of 1.5 per cent agar containing 2.0 ml. specific rabbit antiserum against bovine IgG. The precipitation reaction proceeded for 24 hours at laboratory temperature. Included in each series were standards of various IgG concentrations.

**Antiserum**

Antiserum to bovine serum proteins was obtained from the Institute of Sera and Vaccines, Prague. Specific antiserum to bovine IgG was obtained by immunization of rabbits with pure bovine IgG obtained by precipitation of the blood serum with 33 per cent saturated ammonium sulphate and subsequent fractionation on DEAE cellulose. The antiserum obtained was saturated with non-specific immunoglobulins IgM and IgA.

**Results**

The precolostral sera of calves from both groups of vaccinated cows showed HI titres of 40 to 1280 against PI-virus and of 4 to 128 against *M. bovirhinis*. The precolostral sera of control calves showed HI titres of 40 and 64 to 128 against PI-3 virus and *M. bovirhinis*, respectively. The results are presented in Table 1. After separation of the precolostral calf sera on Sephadex G-200, the elution curves showed only two peaks in contrast to adult sera. The second peak, which in adult sera contained IgG, was either absent or, if present, was only suggested (Fig. 1).

Examination of precolostral serum fractions obtained by separation on Sephadex G-200 failed to detect antibody response to *M. bovirhinis*, whereas antibodies to PI-3 virus were demonstrated occasionally even in protein fractions of high molecular weight. The pattern of antibodies obtained by fractionation on Sephadex G-200 is shown in Fig. 2.
When some precolostral serum samples were subjected to immunoelectrophoresis, most of them migrated cathodally giving rise to precipitin lines similar in shape and position to those of IgG in adult animals (Fig. 3).

The same samples were processed by means of quantitative radial immunodiffusion using antisera to bovine IgG and compared with a series of variously diluted adult sera. Almost all the samples were found to contain IgG in various quantities. The results are presented in Table 1.
Portions of precolostral sera of calves from vaccinated cows were pooled to form a representative sample. One part of the sample was separated on Sephadex G-200 and the other part was precipitated with ammonium sulphate, dialysed and separated on a column of DEAE cellulose. Fractions corresponding to individual peaks of the elution curves were examined for IgG content by quantitative radial immunodiffusion. In the fractions obtained by separation on Sephadex G-200, IgG was found in the first peak and between...
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Calf No.</th>
<th>HI antibody titre</th>
<th>IgG content relative to standard for adult animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PL-3</td>
<td>M. bovirhinis</td>
</tr>
<tr>
<td>Group 1: Calves from dams vaccinated with PL-3 virus + M. bovirhinis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K 7944</td>
<td>160</td>
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<tr>
<td></td>
<td>K 238</td>
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<td></td>
<td>K 7978</td>
<td>80</td>
<td>-</td>
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<tr>
<td></td>
<td>25986</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>345</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>4780</td>
<td>-</td>
<td>32</td>
</tr>
<tr>
<td>Group 2: Calves from dams vaccinated with PL-3 virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4227</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>9749</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4141</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>259</td>
<td>160</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>9682</td>
<td>1280</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>9752</td>
<td>-</td>
<td>64</td>
</tr>
<tr>
<td>Group 3: Control calves from non-vaccinated dams</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9887</td>
<td>-</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>4014</td>
<td>-</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>K 42736</td>
<td>40</td>
<td>-</td>
</tr>
</tbody>
</table>

the first and second peaks, whereas examination of the fractions obtained by separation on DEAE cellulose demonstrated the greatest amount of IgG in the first peak and at the end of the elution curve (Fig. 4).

The identification of IgG in the precolostral calf sera was confirmed by its separation from activated Sepharose 2B employed as immunoadsorbent. Elution of the coupled immunoglobulin of precolostral calf serum with two glycine buffers of different pH gave rise to two fractions shown graphically in Fig. 5. After concentration, the immunoglobulin was demonstrated in both fractions by immunoelectrophoresis using specific antiserum to IgG (Fig. 6). The antibody activity against PI-3 virus and *M. bovirhinis* in the first fraction provided evidence for antibody character of the immunoglobulin isolated in this study (Fig. 5).

Fig. 4. Fractionation of blood sera from newborn calves on Sephadex G-200 (upper) and DEAE cellulose (lower). The bars represent IgG concentrations in individual fractions. A = absorbancy; V = eluate volume.
Fig. 5. The elution curve of immunoglobulin of newborn calves from immunoadsorbent. Black and white bars represent antibodies to PI-3 virus and *M. bovirhinis*, respectively. A = absorbancy; V = eluate volume

Fig. 6. Immunoelectrophoresis of immunoglobulin fractions of blood sera from newborn calves obtained by elution from immunoadsorbent. Upper: fraction 1; lower: fraction 2. Trough: antiserum against bovine IgG

**Discussion**

To elucidate the ontogenesis of immune response of calves in relation to their immunological potential, it is necessary to establish the character of immunoglobulins of their blood sera immediately after birth and during early neonatal life.

From this point of view, the difference between adult cows and calves before ingestion of colostrum lies particularly in the blood serum content of immunoglobulins and antibodies.

The experiments reported in the present study demonstrated specific antibodies to PI-3 virus and *M. bovirhinis* in precolostral sera of calves from both vaccinated and non-vaccinated dams. There were no significant differences in antibody content between the three groups of animals employed. The presence of antibodies in the blood sera of the controls can be accounted for by natural infection of the dams before parturition.

In agreement with some writers (Kniazeff et al., 1969; McCoy et al., 1967; Merriman, 1971) the precolostral calf sera in the present study were found to contain immunoglobulin which was identified as IgG by immunoelectrophoresis and quantitative radial immunodiffusion.

Antigenic relatedness of immunoglobulin of calf precolostral sera to IgG of adult animals, and antibody activity of the former were demonstrated by its separation from activated Sepharose 2 B employed as immunoadsorbent.
The IgG content of precolostral serum varied considerably from calf to calf, ranging from 1/35 to 1/1000 of that found in adult animals. No correlation, however, was found between antibody titre and immunoglobulin content.

The heterogeneity of neonatal calf IgG is perceptible from its distribution in fractions obtained by separation on Sephadex G-200 and DEAE cellulose. This finding was substantiated by the immunoadsorbent elution curve representing two fractions of IgG.

While serological examination of unconcentrated fractions obtained by separation on Sephadex G-200 failed to detect antibodies to *M. bovirhinis*, specific antibodies to PI-3 virus were demonstrated in individual fractions in a wide range of the elution curves. These results suggest that the components of precolostral IgG differ not only by mobility in the electric field but also by molecular size. Similar conclusions were reported by ŠPROKŠOVÁ et al. (1969) for precolostral sera of piglets. Similarly to the results reported by PORTER (1971), our examinations of calf sera failed to reveal IgA. The presence of IgM in precolostral calf sera is still open to question, although MERRIMAN (1971) demonstrated the latter by immunoelectrophoresis.

An important point in respect of further prospects of the prevention and control of respiratory infections in calves is the origin of immunoglobulins in their precolostral sera. It should be determined whether these antibodies are obtained by transplacental transfer of maternal antibody or are produced actively by the foetus. This problem will be the subject of another study.

**Summary**

Precolostral sera of 12 calves from dams vaccinated with either live parainfluenza 3 (PI-3) virus plus live Al(OH)₃-adsorbed *Mycoplasma bovirhinis* vaccine or PI-3 virus alone were found to contain immunoglobulin of antibody character. Its content in the precolostral sera varied from calf to calf ranging from 1/35 to 1/1000 of that found in adult animals. The precolostral immunoglobulin separated by means of immunoadsorption was found to be antigenically related to IgG of adult animals. The patterns of elution curves of precolostral calf sera from Sephadex G-200 and DEAE cellulose as well as the IgG separation from immunoadsorbent suggest that precolostral IgG has heterogeneous properties in respect of molecular weight and electrophoretic mobility.

**Zusammenfassung**

Serumantikörper und Immunglobuline bei neugeborenen Kälbern

Résumé

Anticorps sériques et immunoglobulines chez des veaux nouveaux-nés

Les sérums de 12 veaux, dont les mères avaient été immunisées avec un vaccin Parainfluenza 3 (PI-3) vivant et un vaccin adsorbé avec Al(OH)₃ de Mycoplasma bovirhinis ou avec un virus PI-3 seul, présentaient des immunoglobulines à caractères d’anticorps avant l’absorption de colostrum. Le titre des sérums avant l’absorption de colostrum variait selon le groupe des veaux et se situait entre 1/35 et 1/1000 de celui des animaux adultes. L’immunoglobuline précolostrale isolée par immunoadsorption possédait un caractère antigénique commun avec l’IgG des animaux adultes.

Sur la base des courbes d’éluition de Sephadex G-200 et DEAE-Cellulose, et de l’isolement d’IgG avec un immunadsorbant, l’IgG précolostrale s’est révélée hétérogène en ce qui concerne son poids moléculaire et sa mobilité électrophorétique.

Resumen

Seroanticuerpos e inmunoglobulinas en terneros recién nacidos

Los sueros sanguíneos de 12 terneros, cuyas madres habían sido inmunizadas bien con una vacuna de virus vivo parainfluenza 3 (PI-3) y Mycoplasma bovirhinis vivo, adsorbido a Al(OH)₃, o con virus PI-3 solo, contenían, antes de ingerir calostro, inmunoglobulina con carácter de anticuerpos. El título en los sueros recogidos antes de la ingestión de calostro variaba según los grupos de terneros y oscilaba entre 1/35 y 1/1000 parte de los de los animales adultos. La inmunoglobulina precostral isólemente mediante inmunoadsorción presentaba un parentesco antigénico con la IgG de los animales adultos. A la vista del modelo de curvas de elución en Sefadex G-200 y celulosa DEAE, así como en vista del aislamiento de la IgG de un inmunadsorbente, se vio que la IgG precostral se comporta heterogéneamente con respecto al peso molecular y el movimiento electroforético.

References

MENŠÍK, J. et al., 1970: Výzkum etiologie, diagnostiky a prevence virových respiračních a střevních infekcí skotu. Project report, Veterinary Research Institute, Brno.


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