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# RADIOIMMUNOLOGICAL DEMONSTRATION OF ANTIBODIES IN GNOTOBIOTIC PIGLETS INFECTED WITH MYCOPLASMA HYORHINIS

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**Abstract**

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Antibody response of gnotobiotic piglets infected with *Mycoplasma hyorhinis* was investigated by radioimmunoassay. The synthesis of antibodies in vitro was demonstrated in the spleen, trachea, salivary gland and mesenteric lymph nodes, but not in the lung and nasal mucosa. No evidence was obtained to suggest a direct relation between the incidence of antibodies in the blood serum and in the organs under study. The serum antibodies were, for the most part, of the IgM class.

*Gnotobiotic piglets, proteosynthesis, Mycoplasma hyorhinis, immunoglobulins IgG, IgM, IgA, radioimmunological analysis.*

Infections of the respiratory tract are one of the main problems in pig herds under intensive husbandry conditions. A major role in these infections is played by *Mycoplasma hyorhinis* and *Mycoplasma hyopneumoniae* which are responsible for considerable patho-anatomical and clinical changes particularly among piglets. If gnotobiotic piglets are infected intranasally with a pathogenic strain of *M. hyorhinis* within one week of birth, about 50 per cent of them develop clinical disease with signs of lameness and prostration and with a fatal outcome in some cases. Post-mortem examination generally reveals polyserositis, arthritis and pneumonia (Goiš et al., 1971; Goiš and Kuksa, 1974).

The presence of serum antibodies in gnotobiotic piglets infected with *M. hyorhinis* was demonstrated by Goiš et al. (1972) with the latex-agglutination test between 14 and 35 days after infection. On the other hand, no antibodies were detected by the metabolic inhibition test. Immunoelectrophoresis revealed precipitation lines suggestive of both IgM and IgG.

Radioimmunoassay (RIA) is remarkably sensitive and makes it possible not only to assess the total titre of antibodies, but also to differentiate them as to their class specificity (Rodák et al. 1978, 1983; Taylor and Howard 1980). Since IgM antibodies are produced mainly in the early phase of infection, their determination in the blood serum permits rapid diagnosis of acute infections soon after their onset.

The object of the present study was radioimmunological demonstration of antibodies in gnotobiotic piglets with reference to the possibility of localizing their production in the body and of determining their class specificity.

## Materials and Methods

### Infection

Eight colostrum-deprived gnotobiotic piglets reared in incubators were inoculated intranasally with $1 \times 10^9$/ml of *M. hyorhinis* strain Š 381 at 6 days of age and killed at 12, 19, 22 or 28 days p.i. Bacteriological examination for the presence of *M. hyorhinis* by culture was carried out quantitatively in the lung, liver, kidney and spleen, whereas the other organs were evaluated only as *M. hyorhinis*-positive or negative.

**Table 1**
**Demonstration of antibodies in gnotobiotic piglets infected with Mycoplasma hyorhinis**

| Piglet. No. | Day after infection | Patho-anatomical findings | Organ culture | Antibody titre | |
|---|---|---|---|---|---|
| | | | | Organ culture | Serum |
| 3 | 12 | first--degree pneumonia | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 1:10<br>0<br>1:5<br>0<br>0<br>1:20 | 0 |
| 7 | 12 | first--degree pneumonia | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 0<br>0<br>0<br>0<br>0<br>0 | 0 |
| 0 | 19 | second--degree pneumonia | spleen<br>lung<br>salivary gland<br>lymph nodes<br>nasal mucosa<br>trachea | 0<br>0<br>1:5<br>0<br>0<br>1:10 | 1:640 |
| 4 | 19 | second--degree pneumonia | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 1:20<br>0<br>0<br>0<br>0<br>0 | 1:320 |

### Cultivation of Organ Fragments

Spleen, lung, salivary gland, nasal mucosa, mesenteric lymph node and tracheal tissues of the experimental piglets were minced into 1- to 2-mm fragments, washed in several changes of Trowel's medium and placed, 25 to a plastic lattice, in 3-ml Petri dishes containing 1.2 ml of culture medium which was essentially that of trowel supplemented with inactivated foetal calf serum (1 %) and penicillin and streptomycin. The cultures thus prepared were incubated in an atmosphere of 95 % $O_2$ and 5 % $CO_2$ at 37°C for 24 hours.

After cultivation, the tissue fragments were frozen, thawed, homogenized and then separated from the culture medium by centrifugation at 3500 r.p.m. for 15 minutes. The organ culture fluid was dialysed against phosphate buffered saline, pH 7.2, for 48 hours and concentrated to the original volume of the medium.

### Radioimmunological Determination of Antibodies in the Serum and Organ Cultures

The solid-phase RIA was the same as described in previous studies (Hamp l et al. 1978, 1981). Polystyrene microtubes were used as the solid phase. Antigen was incubated for 16 hours and the organ culture fluids and the blood sera for 3 hours at room temperature. The second antibody, i.e. $^{125}$I-labelled rabbit anti--swine gamma-globulin fraction having a radioactivity of circa 20 000 counts per minute (cpm)/0.050 ml, was incubated also for 3 hours. Blood sera and organ culture fluids from non-infected piglets raised in incubators were used as controls.

Cont. from Table 1

| Piglet No. | Day after infection | Patho-anatomical findings | Organ culture | Antibody titre | |
|---|---|---|---|---|---|
| | | | | Organ culture | Serum |
| 2 | 22 | third--degree pneumonia | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 0<br>0<br>0<br>0<br>0<br>0 | 1:10 |
| 5 | 22 | second--degree pneumonia | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 0<br>0<br>0<br>0<br>0<br>0 | 0 |
| 1 | 28 | third-degree pneumonia pleuritis pericarditis polyarthritis peritonitis | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 1:20<br>0<br>1:10<br>0<br>1:10<br>0 | 1:5120 |
| 6 | 28 | third--degree pneumonia | spleen<br>lung<br>salivary gland<br>nasal mucosa<br>lymph nodes<br>trachea | 0<br>0<br>0<br>0<br>0<br>0 | 1:10 |

Radioimmunological Determination of Class--Specific IgG, IgM and IgA Antibodies in the Serum

To determine class-specific antibodies against *M. hyorhinis*, $^{125}$I-labelled anti-swine IgG, IgM and IgA antibodies, isolated from class-specific rabbit antisera, were employed. These labelled antibodies reacted according to their specificity only with heavy chains ( $\gamma, \mu, \alpha$) of immunoglobulins of the corresponding class. The specificity of the labelled antibodies was confirmed by radioimmunoelectrophoresis (R o d á k 1978).

## Results

Antibodies in the sera of colostrum-deprived gnotobiotic piglets raised in incubators and inoculated intranasally with *M. hyorhinis* were first detected by RIA on the 19th day after inoculation in both animals examined (Table 1). Of two piglets sacrificed on the 22nd day after inoculation only one showed an antibody titre of 1:10 and the other one had no antibodies.

A very high titre of serum antibodies was demonstrated in piglet No. 1 with marked clinical signs of the disease, sacrificed on the 28th day after inoculation. At post-mortem this piglet had the most extensive patho-anatomical changes (polyserositis) and the infectious agent was isolated from its organs by culture. The concentrations of *M. hyorhinis* in its organs was higher than in the other animals (Table 2).

Class-specific (IgG, IgM and IgA) antibodies against *M. hyorhinis* antigens were detected in the experimental piglets as early as the 12th day after infection (Table 3). The first to appear were IgM antibodies which persisted throughout the observation period and showed the highest titres. They were followed by IgG antibodies whose titres were generally lower. The IgA antibody titres were the lowest except in piglet

Table 2

**Recovery of Mycoplasma hyorhinis from the organs of gnotobiotic piglets after experimental infection**

| Piglet No. | Day after infection | Organs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nasal mucosa | Trachea | Tonsil | Pericardium | Lung | Liver | Spleen | Kidney | Joints | |
| | | | | | | | | | | front | hind |
| 3 | 12 | + | + | + | 0 | $10^4$ | 0 | 0 | 0 | + | + |
| 7 | 12 | + | + | + | 0 | $10^4$ | 0 | 0 | 0 | 0 | 0 |
| 0 | 19 | + | + | + | 0 | $10^1$ | 0 | 0 | 0 | 0 | + |
| 4 | 19 | + | + | + | 0 | $10^7$ | 0 | 0 | 0 | 0 | + |
| 2 | 22 | + | + | + | + | $10^6$ | $10^1$ | 0 | 0 | + | + |
| 5 | 22 | + | + | + | 0 | $10^4$ | $10^1$ | $10^1$ | $10^1$ | + | 0 |
| 1 | 28 | + | + | + | + | $10^8$ | $10^3$ | $10^3$ | $10^3$ | + | + |
| 6 | 28 | + | + | + | + | $10^6$ | $10^1$ | $10^1$ | $10^1$ | + | + |

Table 3

**Demonstration of class-specific antibodies in the blood sera of gnotobiotic piglets infected with Mycoplasma hyorhinis**

| Piglet No. | Days after infection | Titres | | |
|---|---|---|---|---|
| | | IgG | IgA | IgM |
| 3 | 12 | 0 | 0 | 0 |
| 7 | 12 | 0 | 0 | 0:60 |
| 0 | 19 | 1:1280 | 1:20 | 1:5120 |
| 4 | 19 | 1:80 | 1:80 | 1:2560 |
| 2 | 22 | 0 | 1:20 | 1:160 |
| 5 | 22 | 0 | 0 | 1:10 |
| 1 | 28 | 1:2560 | 1:2560 | 1:5120 |
| 6 | 28 | 1:80 | 0 | 1:160 |

No. 1 which showed most pronounced patho-anatomical changes, namely pleuritis, pericarditis, peritonitis, and the most extensive pneumonic lesions.

Investigation of the synthesis of antibodies in the organ cultures demonstrated the lack of a direct relation between their incidence in the blood serum and in the organs. The antibodies were synthesized in the spleen, trachea, salivary gland and occasionally in the mesenteric lymph nodes, but in no instance in the lung or in the nasal mucosa (Table 1).

## Discussion

The results of antibody response to *M. hyorhinis* obtained here by RIA agree essentially with those reported by G o i š et al. (1972), but the method used in the present study is more sensitive and more rapid to perform.

For the determination of antibodies against mycoplasma antigen in the organ culture, use was made of $^{125}$ I-labelled anti-swine gamma-globulin antibodies, isolated from hyperimmune rabbit serum. These antibodies were not class-specific and reacted not only with heavy chains of IgG, but also with light chains of all immunoglobulin classes.

The finding that the titres of class-specific IgM antibodies in the blood sera were, for the most part, higher than those of IgG and IgA suggests a primary antibody response and a higher avidity of IgM antibodies for the antigen. This phenomenon was confirmed by repeated examination. It may be presumed that where class-specific antibodies are used, reaction occurs only with one class of bonded immunoglobulins. This results in reduced nonspecific activity binding in control samples, an advantage in the evaluation of experimental samples. The determination of class-specific antibodies thus proved to be of value not only in the elucidation of the course and dynamics of antibody response of infected piglets, but also as a factor contributing to a higher sensitivity of antibody detection by RIA.

The origin of the serum antibodies is obviously to be ascribed to lymphoid tissues other than those followed in the present study. An explanation for the high level of antibodies on day 28 in piglet No. 1 may be seen in the development of vast populations of plasma cells and immunoglobulin-secreting cells in the bone marrow, other lymph nodes and possibly other tissues.

## Radioimunologický průkaz protilátek u bezmikrobních selat infikovaných *Mycoplasma hyorhinis*

Radioimunologickou technikou byla sledována protilátková odpověď bezmikrobních selat infikovaných *Mycoplasma hyorhinis*. Syntéza protilátek in vitro byla

prokázána ve slezině, trachee, slinné žláze a mízní uzlině, přičemž nebyla zjištěna v plicích a v nosní sliznici. Přímý vztah mezi výskytem protilátek v krevním séru a sledovaných orgánech nebyl prokázán. Protilátky, které byly zjištěny v séru, byly převážně třídy IgM.

Радиоиммунологическое определение антител безмикробных поросят, инфицированных *Mycoplasma hyorhinis*

Радиоиммунологическим методом проводились исследования антител у безмикробных поросят, инфицированных *Mycoplasma hyorhinis*. Синтез антител в пробирке был установлен в селезенке, дыхательном горле, слюнных железах и лимфатических узлах. Они не были выявлены в легких и слизистой носа. Прямое отношение между наличием антител в кровяной цыворотке и исследуемых органов не было установлено. Антитела были установлены в сыворотке и преимущественно относились к категории IgM.

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