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***Brief Communication***

# AN INDIRECT HAEMAGGLUTINATION TEST FOR DETECTION OF ANTIBODIES AGAINST MYCOPLASMA HYOPNEUMONIAE USING FORMALINIZED TANNED SWINE ERYTHROCYTES

The indirect haemagglutination (IHA) test has proved to be a sensitive serological test for detection of antibodies against various mycoplasma species, e. g. Mycoplasma hyopneumoniae (M. suipneumoniae) (*Goodwin et al.* 1969, *Lam & Switzer* 1971). In this test fresh erythrocytes were used which have the disadvantage of being unstable during prolonged storage.

Washed and sonicated suspensions of M. hyopneumoniae ("J"-strain) were used in the present experiment. The organisms were grown in Medium FF recommended by *Friis* (1971), with the exception that swine serum was substituted for horse serum. The details concerning antigen production, formalinization, tanning, sensitization of erythrocytes, and performance of the IHA test will be described (*Holmgren,* in press). Formalinized and antigen-coated cells retain their stability for at least 8 weeks when stored at 4 °C and — 70 °C. Investigations concerning long-time storage of the cells are in progress. Formalinized erythrocytes are superior, because a large batch of cells can be sensitized with the antigen and stored in small aliquots ready for use.

Table 1. Presence of antibodies to M. hyopneumoniae by IHA test in sera of slaughter-pigs with enzootic pneumonia.

| IHA titre* | Number of pigs reacting | Relative % of pigs reacting |
|---|---|---|
| ≤ 1:16 | 11 | 11.2 |
| 1:32 | 12 | 12.3 |
| 1:64 | 12 | 12.3 |
| 1:128 | 16 | 16.3 |
| 1:256 | 16 | 16.3 |
| 1:512 | 12 | 12.3 |
| 1:1024 | 7 | 7.1 |
| 1:2048 | 7 | 7.1 |
| ≥ 1:4096 | 5 | 5.1 |
| Total | 98 | |

* Titres are expressed as reciprocal of the serum dilution.

Table 2. Presence of antibodies to M. hyopneumoniae by IHA test in sera of slaughter-pigs derived from herds believed to be free from enzootic pneumonia.

| Herd no. | Number of investigated pigs | Number of sera with IHA titre $\geq$ 1:32 |
|---|---|---|
| 1 | 2 | 0 |
| 2 | 7 | 1 |
| 3 | 2 | 0 |
| 4 | 18 | 0 |
| 5 | 11 | 0 |
| 6 | 21 | 1 |
| Total | 61 | 2 |
| SPF | 17 | 0 |

Individual blood samples were taken at 2 different abattoirs from slaughter-pigs affected with enzootic pneumonia.

Six herds were selected with the aid of the Pig Health Control System at Skara, Sweden (*Hornvall & Bäckström* 1971). They were all so-called integrated herds* of different size. The numbers of sows (including gilts) varied from 2 to 45, with an average of 15. The evidence upon which the herds were considered to be free from enzootic pneumonia was that none of the pigs slaughtered during the last 2 years had shown any pneumonic lesions at slaughter. Histologic examinations of the apical and cardiac lobes in pigs from which negative control sera were taken proved to be negative. In addition, 17 SPF sera were kindly supplied by Dr. P. Madsen, the Danish Meat Research Institute, Roskilde, Denmark. In the IHA test using formalinized cells a rabbit antiserum prepared against M. hyopneumoniae reacted to a titre of 1:16.384. Rabbit antiserum against M. hyorhinis reacted to a titre less than 1:8. The rabbit antisera had previously been absorbed with freeze dried liquid culture media, inactivated and absorbed with packed swine erythrocytes. Table 1 illustrates IHA titres in sera from 98 pigs with pneumonic lesions. Eighty-eight % of the pigs had antibody titres $\geq$ 1:32. Table 2 shows IHA titres in sera from 61 slaughter-pigs from 6 herds believed to be free from enzootic pneumonia. Two pigs (3.2 %) reacted to a titre $\geq$ 1:32. Of 17 SPF-pigs all were negative. The results lend strong support to the assumption that the IHA test measures specific antibodies against M. hyopneumoniae. The correlation between pneumonia and serologic findings is good. For

* Fatteners produced only from the own stock herd.

various reasons it cannot be expected to be absolute, because agents other than M. hyopneumoniae can give pneumonic lesions resembling those associated with enzootic pneumoniae (*Jericho* 1968). Moreover, it is not unlikely that some antigenic heterogeneity exists between different strains of M. hyopneumoniae in the field. This might decrease the sensitivity of a serologic test when using one single mycoplasma strain as antigen. Three % of the pigs from the integrated herds had IHA titres $\geq$ 1:32. As there is no reason to believe that the tested pigs would not be free from enzootic pneumonia, the result indicates some unreliability of the IHA test. This is not an unexpected finding, as no serologic test is 100 % reliable. In spite of these 3 % false-positive reactions, the IHA test with formalinized erythrocytes may be of value in epidemiologic studies of M. hyopneumoniae infection. The sensitivity and simplicity of the test make it suitable for repeated tests.

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