

Brief Communication

MYCOPLASMA SUIPNEUMONIAE ISOLATED
IN DENMARK

Though several agents may be involved in the etiology of enzootic pneumonia of pigs, recent investigations seem to indicate that the great majority of cases are caused by a *Mycoplasma* species — called *M. suis*pneumoniae in Britain (*Goodwin et al.* 1965) and *M. hyopneumoniae* in the U.S.A. (*Maré & Switzer* 1965).

In Denmark catarrhal lung lesions typical of enzootic pneumonia are frequently found in pigs at meat inspection, and investigations have for some time been carried out in order to identify the mycoplasmas present in lungs of Danish pigs.

From the lungs of one pig an agent has now been recovered which fits in well with the characteristics of *M. suis*pneumoniae. The isolation of this agent was accomplished in a new type of medium, which has proved capable of supporting growth of *M. suis*pneumoniae. The composition of this *medium*-FF is as follows:

Hank's balanced salt solution, 400 ml; double-distilled water, 715 ml; brain heart infusion broth (Difco), 8.5 g; tryptic digest broth (Difco), 6.4 g; lactalbumin hydrolysate (Difco), 2.0 g; yeast extract (Difco), 2.0 g. This mixture is autoclaved, and 35 ml of a freshly prepared extract of Fleischmann's pure dry yeast type 2040, 25 ml 10 % glucose, 300.000 i.u. of penicillin, and 2.5 ml 5 % thalliumacetate added. To this basal medium 25 % sterile horse serum is added. Per 100 ml of final medium 0.4 ml of a 7.5 % NaHCO_3 solution is added. Phenol red is used as pH indicator. Solid medium is prepared with special agar-Noble (Difco) in a 0.9 % concentration. The agar is autoclaved in a 15 % solution (double-distilled water) and added to the medium at 50°C.

For growth inhibition tests, discs were completely soaked with rabbit hyper-immune serum against *M. suis*pneumoniae¹⁾, dried at 37°C, and stored at + 4°C until use.

Metabolic inhibition tests with rabbit hyper-immune serum against *M. suis*pneumoniae¹⁾ were read when a pH decrease of 0.5 had occurred in the control tubes.

¹⁾ Type NCTC 10110 obtained from Dr. R. H. Leach, Mycoplasma Reference Laboratory, Colindale, London.

In July 1969 five lungs of pigs originating from four unknown herds were collected at a Danish slaughter-house. All of these lungs showed lesions typical of catarrhal pneumonia.

Pneumonic lung tissue was carefully minced and suspended in fluid medium in dilutions up to 10^{-7} . After three passages in fluid medium subcultures on solid medium were made.

In the tubes inoculated with material from four of the lungs a rapid decrease of pH was noted, and subcultures on solid medium showed growth of large mycoplasma colonies of the "fried-egg" type (Fig. 1) which were identified as *Mycoplasma*

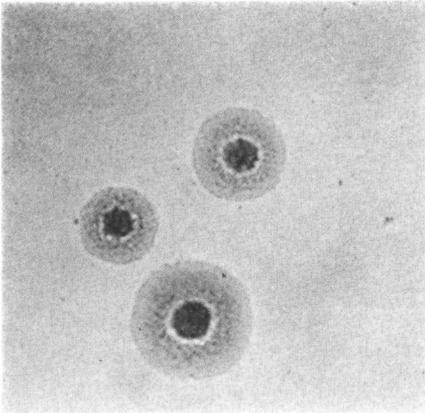


Figure 1. *Mycoplasma hyorhinis* isolated from the lung of a Danish pig with catarrhal pneumonia. After 2 days of incubation on solid medium-FF. $\times 32$.

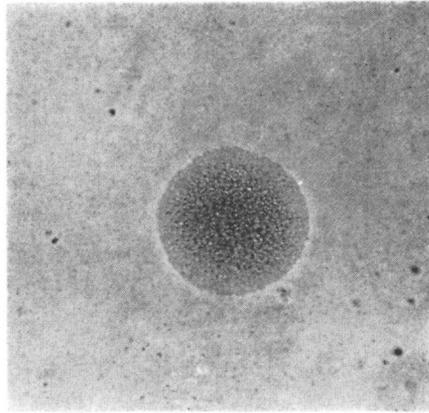


Figure 2. *Mycoplasma suis pneumoniae* isolated from the lung of a Danish pig with catarrhal pneumonia. After 6 days of incubation on solid medium-FF. $\times 32$.

hyorhinis types. The endpoint titers obtained for these four lungs were 10^{-3} , 10^{-4} , 10^{-5} and 10^{-7} .

In the tubes inoculated with material from the fifth lung no growth of *M. hyorhinis* occurred. However, after about one week a slowly progressing decrease of pH was noticed. On subcultures on solid medium, colonies morphologically resembling *M. suis pneumoniae* were visible after 4 days. After one week the size of the colonies ranged up to 200μ and no central nipple was visible (Fig. 2). Growth inhibition test against *M. suis pneumoniae* antiserum showed a 3 mm broad zone of inhibition, and in the metabolic inhibition test with the same antiserum lack of pH shift was recorded up to a dilution of $1/128$. In the primary cul-

tures from the lung material an endpoint titer of 10^{-3} was obtained.

In August 1969 six more lungs were collected, all of which showed lesions of catarrhal pneumonia. From two of these lungs, strains of mycoplasma were isolated which showed the same cultural characteristics as the one described above. Serological identification has confirmed the identity to *M. suis*pneumoniae.

The pathogenicity of Danish isolates of *M. suis*pneumoniae has not yet been ascertained. If, however, their pathogenicity for pigs is confirmed, it is reasonable to assume that this mycoplasma species is the main cause of catarrhal pneumonias in Danish pigs.

The *medium-FF* described in this article is a medium of a new composition. It has been confirmed that it is capable of supporting growth of *M. suis*pneumoniae, but it is not yet known with certainty whether it is quite as well suited for primary isolation of this mycoplasma as the medium described by *Goodwin et al.* It is easier to prepare, however, and it has been established that its capacity of supporting growth varies very little from one batch to the other. For the isolation of other mycoplasmas from pigs, especially *M. hyorhinis*, it has turned out to be excellent.

Niels F. Friis

State Veterinary Serum Laboratory
Copenhagen, Denmark.

REFERENCES

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(Received August 25, 1969).

