# MYCOPLASMAL AND BACTERIAL FLORA IN THE LUNGS OF PIGS

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INTRODUCTION: Apart from Mycoplasma hyopneumoniae which had already been established as the primary etiological agent of enzootic pneumonia of pigs (EPP), some strains of M. hyorhinis are also claimed to induce pneumonia in gnotobiotic piglets when inoculated intranasally at the age of 10 days [2,3]. The aim of this paper is to investigate the mycoplasmal and bacterial flora in porcine lungs with or without pneumonic lesions and evaluate the isolates with respect to their epizootiological significance in EPP in Japan.

MATERIALS & METHODS: Lung materials; A total of 36 lungs with typical gross lesions of EPP was obtained at two abattoirs and in addition lungs were collected at slaughter from 78 pigs derived from 3 pig farms infected with EPP of which 61 pigs had pneumonic lesions and the remaining 17 were macroscopically normal.

Media; BHL-broth or agar used for the isolation of M. hyopneumoniae consisted of Brucella broth (Gibco) 0.58g, Lactalbumin hydrolysate (NBC) 0.2g, distilled water 24 ml, phosphate buffered solution 50 ml, porcine serum 20 ml, 25%(w/w) extract of baker's yeast (Nitten) 5 ml, methicillin solution (10mg/ml) 1 ml. Phosphate buffered solution contained the following constituents per liter, NaCl 8.0g, KCl 0.4g, $Na_2HPO_4 \cdot 12H_2O$ 0.15g, $KH_2PO_4$ 0.06g, glucose 1.0g, 0.4% phenol red solution 5 ml. Agar medium was prepared by adding Agarose (Nakarai Chem.) to the broth at the final concentration of 1%. In the primary isolation, BHL-broth supplemented with 0.5% of rabbit antiserum against M. hyorhinis BTS 7 and M. hyosynoviae S16 and 1 mcg/ml of kanamycin sulfate was also used to isolate M. hyopneumoniae selectively. For isolating M. hyorhinis and M. hyosynoviae, M-broth and M-agar [4] were used. Blood and chocolate agar plates which were prepared by supplementing Trypticase soy agar (BBL) with 5% of defibrinated horse blood, were used for examination of aerobic bacteria.

Isolation procedure; A piece of lung material was stamped on blood and chocolate agar plates and then 10% homogenates were made with BHL-broth. After centrifugation at 1,000 rpm for 5 min., 0.3 ml of the supernatant fluid was inoculated into 2.7 ml of selective and non-selective BHL-broth and M-broth making ten-fold dilutions in each medium up to $10^{-6}$. Tubes were incubated at 37°C. After 3 days incubation, cultures from each dilution of M-broth were transfered to two M-agar plates. One was incubated aerobically and the other was incubated at strict anaerobic condition. The latter facilitates the selective isolation of M. hyosynoviae [4]. Tubes containing BHL-broth were incubated for up to 50 days and when the growth was indicated by pH shift of the medium, passages were made immediately from the highest dilution of inoculum showing growth into BHL-broth without selective agents making ten-fold dilutions up to $10^{-5}$.

Identification of isolates; Mycoplasmas were identified by growth or metabolism inhibition test and and bacteria were according to Cowan [1].

RESULTS & DISCUSSION: Results are summarized in Table 1. A total of 114 lungs was subjected to mycoplasmal and bacterial examination. M. hyopneumoniae was isolated from 90 out of 97 lungs (93%) with lesions of EPP and from 6 of 17 macroscopically normal lungs of pigs derived from enzootic pneumonia infected herds. M. hyorhinis and M. hyosynoviae were isolated from 16% (16/97) and 18% (17/97) of pneumonic lesions and from 18% (3/17) and 29% (5/17) of macroscopically normal lungs, respectively. Pasteurella multocida was the principal bacteria harboured in the lung lesions of EPP. Quantitative determination of mycoplasmas revealed that M. hyopneumoniae was isolated at the high inoculum dilution of $10^{-5}$ or $10^{-6}$ in half of the lungs which yielded this organism. Our newly developed BHL-broth supported the constant and relatively rapid growth of M. hyopneumoniae as compared to modified Cambridge medium which was used in the previous study [6].

M. hyorhinis had been isolated from 67% (42/63) of the pneumonic lungs in our previous survey made in 1967 to 1968 [5], and 52% (16/31) in 1970 [6]. At the present investigations lung materials were collected during 1979 to 1981 and the incidence of this organism decreaced to 16% (16/97). These facts may suggest that the role of M. hyorhinis in EPP in Japan is becoming to be insignificant.

As judged by the incidence and the number of organisms isolated from the pneumonic lung lesions, M. hyopneumoniae is considered to be the main etiological agent with epizootiological significance in EPP in Japan.

SELECTED REFERENCES: 1) Cowan, S. T. (1974): Cowan & Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge Univ. Press, Cambridge. 2) Friis, N. F. (1971): Mycoplasma hyorhinis, a causative agent in pneumonia in pigs. Acta Vet. Scand., 12, 116-119. 3) Gois, M. and Kuksa, F. (1974): Intranasal infection of gnotobiotic piglets with Mycoplasma hyorhinis: Differencies in virulence of strains and influence of age on the development of infection. Zbl. Vet. Med., B, 21, 352-361. 4) Ogata, M., Kawamura, S. and Yamamoto, K. (1982): Selective isolation of Mycoplasma hyosynoviae by anaerobic cultivation. Jpn. J. Vet. Sci., in press. 5) Yamamoto, K. and Ogata, M. (1969): Unpublished data. 6) Yamamoto, K., Koshimizu, K. and Ogata, M. (1971): Selective isolation of Mycoplasma suipneumoniae from pneumonic lesions in pigs. Nat. Inst. Anim. Hlth Quart., 11, 168-169.

Table 1. Microflora of pneumonic and apparently normal lungs of pigs

| Organism | Macroscopic lesion YES | NO |
|---|---|---|
| M. hyopneumoniae | 90/97 (93%) | 6/17 (35%) |
| M. hyorhinis | 16/97 (16%) | 3/17 (18%) |
| M. hyosynoviae | 17/97 (18%) | 5/17 (29%) |
| Pasteurella multocida | 32/97 (34%) | 2/17 (12%) |
| Staphylococcus spp. | 3/95 ( 3%) | 1/17 ( 6%) |
| Streptococcus spp. | 3/95 ( 3%) | 0/17 |
| Haemophilus parasuis | 2/95 ( 2%) | 1/17 ( 6%) |
| Bacillus spp. | 2/95 ( 2%) | 0/17 |