Isolation of *Mycoplasma hyosynoviae* from pneumonic lung of swine

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Received 1 September 2009; received in revised form 26 October 2009; accepted 26 October 2009

**Abstract.** The isolation of *Mycoplasma hyosynoviae* from a piglet with severe pneumonia is described. This is the first report of *M. hyosynoviae* isolation in the country. The lung sample where the isolation was made was severely consolidated, suppurative and pleurisy. The pathogenicity of the *M. hyosynoviae* isolated has yet to be determined.

**INTRODUCTION**

*Mycoplasma hyosynoviae* causes acute arthritis in pigs of 10 weeks of age and older. This organism can be isolated from nasal cavities, throat and lungs of carrier swine (Richard et al, PIH 36). *Mycoplasma hyosynoviae* was also found in blood and could be isolated during acute phase of the infection (Hagedorn-Olsen et al, 1999a).

*Mycoplasma hyosynoviae* was identified as early as 1970 by Ross and Karmon who isolated from joints, respiratory tracts, tonsil and lung of pigs. Previously, twelve strains of this *Mycoplasma* were tentatively identified as *Mycoplasma (now Acholeplasma) granularum* due to similar colony morphology and growth characteristics.

A new nomenclature however was proposed as *M. hyosynoviae* after these twelve strains of *Mycoplasma* were found biologically, serologically and electrophoretically different to the prototype strain of *M. granularum*. The name *M. hyosynoviae* was to reflect the species ‘hyos’ and location ‘synoviae’ of the organism of the diseased host (Ross & Karmon, 1970).

Despite most susceptible pigs being above 10 weeks, there is no evidence of age-related resistance to *M. hyosynoviae* infection in pig (Lauritsen et al, 2008). Pigs younger than 10 weeks of age might be protected against *M. hyosynoviae* infection by passive transferred of colostrum immune factors from sows to their offspring as cited by Lauritsen et al. (2008). Maternal antibodies were transferred to the piglet and persisted for approximately 8-12 weeks and after weaning, some pigs became infected and some pigs did not (Hagedorn-Olsen et al, 1999b).

Isolations of *M. hyosynoviae* have been reported in United States of America (Ross & Karmon, 1970), Australia (Furlong et al, 1975), Denmark (Friss et al, 1991) and Spain (Assuncao et al, 2005).

In Malaysia, the first isolation of swine *Mycoplasma* was made in 1985 from lung of a porker and it was identified as *Mycoplasma gallinarum* (Joseph et al, 1988). This paper reports for the first time the isolation of *M. hyosynoviae* from swine in this country.

**MATERIALS AND METHODS**

**Necropsy Report**

Five (5) pigs with respiratory distress and weight loss were received from the private sector by Regional Veterinary Laboratory (RVL), Petaling Jaya. Necropsy report showed that the swine lungs were severely...
consolidated, suppurative and pleurisy suggestive of pneumonia. Other organs were normal.

Sample
A sample of the swine lung was received by the Veterinary Research Institute from RVL Petaling Jaya for *Mycoplasma* cultivation.

Media
Pleuropneumonia-like Organism (PPLO) agar and MH broth were used for the culture of *Mycoplasma*. The PPLO agar was prepared using PPLO broth base and noble agar enriched with fresh yeast extract, glucose, L-arginine, DNA disodium salt and NAD disodium salt. Inactivated swine serum was later added at a final concentration of 15-20% (v/v).

The MH broth, a selective media for the detection of *M. hyopneumoniae* was prepared according to the method of Tan, 1986. The ingredients in this medium consisted of Hank's balanced salt solution, heart infusion broth, Hartley's broth, Lactabumin hydrolysate, fresh yeast extract, glucose, L-arginine, DNA disodium salt and NAD disodium salt. Thallous acetate, bacitracin and vancomycin hydrochloride were incorporated to inhibit growth of fungal and bacterial contaminations. Inactivated swine serum was later added at a final concentration of 20% (v/v).

Isolation Procedures
The lung sample was cut and smeared directly onto PPLO agar and then macerated into MH broth and incubated at 37°C. The agar plate was incubated with 5-10% CO₂ atmosphere in a moist chamber.

Broth suspension was examined daily to detect any changes in colour or visible turbidity. The broth was filtered through 0.45 µm membrane filter (Toyo Roshi Co Ltd Japan) using Swinney head filter holder to eliminate contaminant. To a fresh 9 ml M.H broth, 1 ml of the filtrate was inoculated. The broth was also cultured onto PPLO agar. Blind passages of the broth media with no evidence of growth were carried out three times at the interval of 5-7 days.

After 72 to 96 hours of incubation, the agar plates were checked for typical *Mycoplasma* colonies under a dissecting microscope at 50x magnification. Suspected *Mycoplasma* colonies undergo at least three times of passages onto agar and broth media without antibiotic to ensure its purity.

Biochemical tests which include glucose fermentation, arginine hydrolysis, phosphatase activity, reduction of tetrazolium chloride and formation of film and spot were carried out for the identification of *Mycoplasma* species.

Test medium for arginine was prepared according to Tan (1986). The main ingredients were sterile inactivated pig serum, fresh yeast extract and arginine. Phenol red was later added as an indicator. The pH of the medium was adjusted to 6.8 and the medium was checked with cultures known to give positive and negative reactions.

Hydrolysis of arginine was carried out with essential control procedure. Two media with different incubation condition were used as controls in this study, basal medium without test substrate and basal medium with the test substrate respectively. The first medium were incubated along with the test where as the later were incubated without inoculation.

RESULT AND DISCUSSION
The PPLO agar plate showed typical ‘fried egg’ colony of *Mycoplasma* spp. after 72 to 96 hours incubation. MH broth showed turbidity indicating growth of *Mycoplasma* spp. However, there was no acidification of the medium suggesting that the isolate was not *M. hyopneumoniae* because *M. hyopneumoniae* is a glucose fermenter. The growth of this *Mycoplasma* was obtained within a week and its colony morphology on the PPLO agar also suggesting that it was a not *M. hyopneumoniae* as *M. hyopneumoniae* does not form the “fried eggs” colonies typical of a majority of mycoplasmas and the propagation in liquid media is very slow, taking several weeks
(Fisher, 1995). Tully & Whitcomb (1979) also reported that *M. hyopneumoniae* is one of the several mycoplasma species that still cannot be grown on agar during primary isolation.

Friss (1975b) reported that *M. hyopneumoniae* is among the species of mycoplasmas which is difficult to propagate due to its fastidious culture requirements and the extremely slow growth of *M. hyopneumoniae*, often resulting in overgrowth by other mycoplasmas colonizing the respiratory tracts of pigs (Holko et al., 2004).

The *Mycoplasma* spp. was identified as *M. hyosynoviae* on the basis of their biochemical reactions (Table 1). This *Mycoplasma* hydrolyses arginine and produces film and spot. The pH of arginine was found shifted to 7.6, indicating an alkaline shift.

These results were in agreement with Farrington & Switzer (1976) who reported that biochemical identification of *M. hyosynoviae* is based on the utilization of arginine which produces an alkalization of the broth medium and causes distinct film and spot reaction.

This *Mycoplasma* spp. is non-glucose fermenter. It did not reduce tetrazolium chloride or produce phosphatase. These results were consistent to that of the species reported in Ross & Karmon (1970) and Holt et al. (1994).

<table>
<thead>
<tr>
<th>TESTS</th>
<th>OBSERVATION</th>
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<tr>
<td>Glucose fermentation</td>
<td>–</td>
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<tr>
<td>Arginine hydrolysis</td>
<td>+</td>
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<tr>
<td>Formation of film and spot</td>
<td>+</td>
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<tr>
<td>Phosphatase activity</td>
<td>–</td>
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<tr>
<td>Reduction of tetrazolium chloride</td>
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In the beginning, this arginine-utilizing *Mycoplasma* showed poor growth on the PPLO agar. However, after several passages, their growth improved. This result suggests that *M. hyosynoviae* requires longer duration to adapt on PPLO agar. The poor growth of *M. hyosynoviae* on agar medium is possibly attributed to the absence of gastric mucin in the PPLO agar. Ross and Karmon (1970) reported that *M. hyosynoviae* grew poorly when gastric mucin was omitted but with the addition of mucin to the medium, growth of the organism improved. Ogata et al. (1982) also reported that for selective isolation of *M. hyosynoviae*, an anaerobic cultivation, in the condition of 5% CO₂ in N₂ is preferred. This *Mycoplasma* was reported to require sterols for growth (Ross & Karmon, 1970). In this study, 15-20% pig serum has been incorporated in the PPLO agar and the *Mycoplasma* was proven to grow in the agar. However, the growth of this *Mycoplasma* without sterols was not investigated in this study.

Many authors have reported the isolation of *M. hyosynoviae* from joints and tendon sheath but very few reported the isolation of this species from pneumatic lung (Furlong & Turner, 1975 et al., Friis, 1991). *Mycoplasma hyosynoviae* is not known to be primary causes of diseases in the respiratory track (Richard et al., PH36). The role of this organism, if any, in causing the pneumatic, suppurative lung is still unclear. Taylor-Robinson & Dinter (1968) reported that, it is conceivable that damaged respiratory tract tissue could be the debilitating factor that made it susceptible to colonization by saprophytic or ‘unrelated’ mycoplasma (Joseph et al., 1988).

It has been well documented that a single *Mycoplasmal* infection normally produces mild respiratory symptoms, but the disease may become severe if aggravated by secondary factors such as bacteria, viruses, and poor housing conditions (Fris, 1975a). Hagedorn-Olsen et al. (1999a) suggested that development of arthritis may depend on age, immunity, virulence factor, stress or lowered general resistance of the animal.

Friis et al. (1991) suggested that the tonsil is a reservoir for *M. hyosynoviae* and is probably the location of choice for an easy demonstration of the presence of this *Mycoplasma* in a pig herd. *Mycoplasma hyosynoviae* may be present in the pig herd without producing evidence of arthritis.
A later report however, showed that *M. hyosynoviae* has special affinity for joint tissue and may be able to cause arthritic disease (Kobich & Friss, 1996). The tonsillar and pharyngeal mucosa of adult swine are frequent site of chronic infection with *M. hyosynoviae* and this probably is the principle way it is maintained in a herd.

In the present work, *M. hyopneumoniae* was the initial suspected causative agent for the respiratory distress. *Mycoplasma hyopneumoniae*, however was not isolated. Although *M. hyopneumoniae* has been reported to be able to colonize the ciliated epithelial cells of the lower respiratory tract, it does not invade lung tissue (Thacker, 2007). This could be one of the reasons for the failure to isolate *M. hyopneumoniae* from the lung. However, the role of the isolate in lungs has yet to be documented elsewhere. Therefore, a comprehensive study on the pathogenicity of *M. hyosynoviae* in lungs and its prevalence in Malaysia is necessary to help in the better understanding of its role in inducing disease and the economic impact that it may have on swine industry.

**Acknowledgements.** The authors would like to thank Dato’ Dr Abdul Aziz Jamaluddin, Director General of Department of Veterinary Services Malaysia, for his permission to publish this paper, Dr Kamarudin Md Isa, Director of Research Division, Department of Veterinary Services Malaysia, Dr Ramlan Mohamed Director of Veterinary Research Institute, Ipoh for the support, guidance and encouragement. The authors also would like to thank Dr Hii Ding Ong from Rhonema Malaysia Sdn Bhd and Dr Nurul Ain from Regional Veterinary Laboratory, Petaling Jaya for the technical information provided.

**REFERENCES**


