Tropical Biomedicine 26(3): 341–345 (2009)

Isolation of *Mycoplasma hyosynoviae* from pneumonic lung of swine

Dahlia, H., Tan, L.J., Zarrahimah, Z. and Maria, J.
Veterinary Research Institute, 31400 Ipoh Perak, Malaysia
Email: dahlia@jphvri.gov.my
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 electrophorethically 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 colostral 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% CO_2 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 alkalinization 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 1. Biochemical tests and reactions for the identification of *M. hyosynoviae*

TESTS	OBSERVATION
Glucose fermentation	–
Arginine hydrolysis	+
Formation of film and spot	+
Phosphatase activity	–
Reduction of tetrazolium chloride	–

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% CO_2 in N_2 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 pneumonic 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.*, PIH 36). The role of this organism, if any, in causing the pneumonic, 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 (Friis, 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 herds without producing evidence of arthritis

(Hagedorn-Olsen *et al*, 1999a; Richard, PIH 36). 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

Assuncao, P., Fec, D.L., Kokovic, B., Gonzalez, O. & Poveda, J.B. (2005). The occurrence of Mycoplasma in the lungs of swine in Gran Canaria (Spain). *Veterinary Research Communication* **29**(6): 453-462.

Farrington, D.O. & Switzer, W.P. (1976). Mycoplasmas and Diseases of Swine. Laboratory Diagnosis of Mycoplasmosis in Food Animal. A special report of the Mycoplasmosis Committee American Association of Veterinary Laboratory Diagnosticians, pg. 72-77.

Fisher, O. (1995). Enhancement of propagation of *Mycoplasma hyopneumoniae* by culture a biphasic medium. *Acta Veterinaria Brno* **64**: 243-247.

Friis, N.F. (1975a). Cytoplasm's of the swine- a review. *Nordisk Veterinær Medicin* **27**(6): 329-336.

Friis, N.F. (1975b). Some recommendations concerning primary isolations of *Mycoplasma suipneumoniae* and *Mycoplasma flocculare*. *Nordisk Veterinær Medicin* **27**: 337-339.

Friis, N.F., Ahrens, P. & Larsen, H. (1991). *Mycoplasma hyosynoviae* isolation from the upper respiratory tract and tonsils of pigs. *Acta Veterinarian Scandinavia* **32**(4): 425-429.

Furlong, S.L. & Turner, A.J. (1975). The isolation of *Mycoplasma hyosynoviae* and exposure of pigs to experimental infection. *Australian Veterinary Journal* **51**(6): 291-293.

Hagedorn-Olsen, T., Nielsen, N.C. & Friis, N.F. (1999a). Induction of arthritis with *Mycoplasma hyosynoviae* in Pigs: Clinical response and re-isolation of the organism from body fluids and organs. *Zentralblatt fur Veterinarmedizin*. Reihe A **46**(6): 317–325.

Hagedorn-Olsen, T., Nielsen, N.C., Friis, N.F. & Nielsen, J. (1999b). Progression of *Mycoplasma hyosynoviae* infection in three pig herds. Development of tonsillar carrier state, arthritis and antibodies in serum and synovial fluid in pig from birth to slaughter. *Zentralblatt fur Veterinarmedizin*. Reihe A **46**(9): 555-564.

Holko, I., Urbanova, J., Holkova, T. & Kmet, V. (2004). Diagnostics of main bacterial agents of porcine respiratory diseases complex (PRDC) using PCR detection of *Mycoplasma hyopneumoniae*. *Veterinarni Medicina* **49**(2): 35–41.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T. & William, S.T. (1994). *Bergey's Manual of Determinative Bacteriology*. Ninth Edition. William and Wilkins, Maryland USA, pg. 705-717.

Joseph, P.G., Tan, L.J., Sivanandan, S.P., Jamnah, O., Cottew, G.S. & Yeats, F. (1988). Mycoplasma infections of animals in Malaysia with emphasis on the isolation and identification of the etiological agents. *Tropical Biomedicine* **5**: 167-177.

Kobich, M. & Friis, N.F. (1996). Swine mycoplasmoses. *Revue Scientifique et Technique* **15**(4): 1569-1605.

Lauritsen, K.T., Hagedorn-Olsen, T., Friis, N.F., Lind, P. & Jungersen, G. (2008). Absence of strictly age-related resistance to *Mycoplasma hyosynoviae* infection in 6-week-old pigs. *Journal of Veterinary Microbiology* **130**(3-4): 385-390.

Ogata, M., Kawamura, S. & Yamamoto, K. (1982). Selective isolation of *Mycoplasma hyosynoviae* by anaerobic cultivation. *Japanese Journal Veterinary Science* **44**: 725-733.

Richard, R., Michael, H. & Richard, L.W. Swine Arthritis. Herd Health, Purdue University, Cooperative Extention Service, West Lafeyette, Indiana. Pork Industry Handbook (PIH) **36**.

Ross, R.F. & Karmon, J.A. (1970). Heterogeneity among strains of *Mycoplasma granularum* and identification of *Mycoplasma hyosynoviae*, sp. n. *Journal of Bacteriology* **103**(3): 707-713.

Tan, L.J. (1986). Diagnosic Techniques on Mammalian Mycoplasma. Laboratory Working Manual, Veterinary Research Institute, Ipoh, Malaysia.

Thacker, E. (2007). Evaluation of diagnostic assays for *Mycoplasma hyopneumoniae:* Their potential role in eradication success, *Research Report Swine Health. Feb*. 20, 2007.

Tully, J.G. and Whitcomb, R.F. (1979). *The Mycoplasma II*, *Human and Animal Mycoplasmas*, Volume Editors. Academic Press, New York. Pg. 134-143.