ISOLATION OF *MYCOPLASMA* SPECIES FROM VARIOUS ANIMAL HOSTS IN MALAYSIA

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ABSTRACT. This paper reports on the isolation of *Mycoplasma* sp from various animal host in Malaysia from 2006 to 2009. Samples received for *Mycoplasma* isolation were submitted to Avian Bacteriology, Veterinary Research Institute, Ipoh. The samples were cultured onto Pleuropneumonia Like Organism (PPLO) agar and broth and were incubated at 37°C with five to ten percent carbon dioxide. Presumptive *Mycoplasma* colonies were passaged onto PPLO agar without antibiotic three to four times for purity. The organisms were then subjected to biochemical tests and growth inhibition test for species confirmation. Identification of *Mycoplasma* species was made based on available specific antisera which focus on the pathogenic *Mycoplasma* of veterinary importance. From July 2006 to 2009, there were 70 isolations were isolated from various animal hosts namely; avian, porcine, caprine and bovine. From avian (poultry and bird), *Mycoplasma synoviae (M. synoviae)* (3), *M. gallisepticum* (3), *M. gallinarum* (9) and *M. columborale* (2) were isolated. From porcine (pig), *M. hyorhinis* (7) and *M. hyosynoviae* (1) were isolated; from caprine (goat and sheep), *Mycoplasma arginini* (17) and *M. agalactiae* (3) were isolated while from bovine (cattle), *Mycoplasma bovis* (2) and *Mycoplasma alkalescens* (2) were isolated. The isolation of *Mycoplasma alkalescens* from cattle is reported for the first time in Malaysia. Other 21 *Mycoplasma* isolations were identified only as *Mycoplasma* species due to unavailability of specific antisera. Further study on the pathogenicity of *Mycoplasma* species, particularly on the newly isolated species, is essential to better understand the roles of the organism in inducing disease in livestock animal.

Keywords: Mycoplasma, pleuropneumonia like organism

INTRODUCTION

Taxonomically, *Mycoplasma* is in the class of Mollicutes which indicate the lack of cell wall (Razin, 1991). The primary habitats of animal mycoplasmas are the mucous surfaces of the respiratory and urogenital tracts and the eyes alimentary canal, mammary glands and the joint in some animals (Razin and Barile, 1985).

Mycoplasma has been recognized for their tendency to interact with other

respiratory pathogens and produce severe disease. *Mycoplasma gallisepticum* for instance, interact with respiratory viruses and *Escherichia coli* and cause chronic respiratory disease (CRD) in chicken. (Kleven, 1998).

The first *Mycoplasma* was isolated from cattle with arthritis and pleuropneumonia in 1898 at Pasteur Institute (Stalheim O.H.V., 1976). In Malaysia, the first *Mycoplasma* was isolated from chicken in 1960 at the Veterinary Research Institute (VRI), Ipoh (Thuraisingam, 1963). Since then, a number of *Mycoplasma* isolations have been made in VRI Ipoh and subsequently reported by Joseph *et al*., 1988 and Zaini *et al*., 2006.

MATERIALS & METHOD

Specimens of suspected cases for Mycoplasmosis from various farms in Malaysia were submitted to VRI for laboratory examination. The specimens such as nasal swabs, trachea and lung were cultured onto PPLO agar and PPLO broth. Agar plates were incubated at 37ºC, with 5% carbon dioxide while PPLO broths were incubated at 37ºC in aerobic condition. The agar plates were examined for any *Mycoplasma*-like colonies, ("fried egg" colony) every three to five days. Visible single colony was subcultured at least three times. PPLO broths were examined for turbidity or pH change, within three to five days. The plates and broth culture were considered negative if no *Mycoplasma*-

TABLE 1: Mycoplasma isolation from July 2006 to Dec 2009

Host species	ISOLATES	2009	2008	2007	2006	Total	Subtotal
Avian	*M. synoviae*	2	1	0	0	3	30
	M. gallisepticum	2	1	0	0	3	
	M. gallinarum	6	1	2	0	9	
	M. columborale	1	1	0	0	2	
	Mycoplasma sp	8	4	1	0	13	
Swine	*M. hyorhinis*	0	4	3	0	7	11
	M. hyosynoviae	0	0	1	0	1	
	Mycoplasma sp	2	0	1	0	3	
Caprine/ Ovine	*M. arginini*	5	4	6	2	17	24
	M. agalactiae	1	0	0	2	3	
	Mycoplasma sp	3	1	0	0	4	
Bovine	*M. bovis*	2	0	0	0	2	4
	M. alkalescens	2	0	0	0	2	
Other	*Mycoplasma* sp	0	1	0	0	1	1
Total		**36**	**18**	**14**	**4**	**70**	**70**

TABLE 2: Biochemical characteristics of Mycoplasma species identified in VRI Ipoh.

Biochemical characteristics	*M. gallisepticum*	*M. synoviae*	*M. gallinarum*	*M. columborale*	*M. hyorhinis*	*M. hyosynoviae*	*M. arginini*	*M. agalactiae*	*M. bovis*	*M. alkalescens*
Requirement of serum for growth	+	+	+	+	+	+	+	+	+	+
Requirement of DNA for growth	-	-	-	-	-	+	-	+	+	+
Sensitivity to digitonin	+	+	+	+	+	+	+	+	+	+
Phosphatase	-	-	-	-	+	-	-	+	+	+
Caseinase	*ND	*ND	*ND	*ND	-	-	-	-	-	-
Glucose	+	+	-	+	+	-	-	-	-	-
Arginine	-	-	+	-	-	+	+	-	-	-
TTC	+	+	+	-	+	-	-	+	+	-
Film and spot formation	-	+	+	-	-	+	-	+	+	-

*ND - Not Done

like colony were seen after 14 to 20 days. *Mycoplasma* colonies were subjected to biochemical tests for species identification. Growth inhibition test using reference strain antisera were carried out for species confirmation.

RESULTS

There were 70 *Mycoplasma* species isolated in VRI from July 2006 to 2009 (Table 1) and biochemical tests were conducted for each *Mycoplasma* species isolated (Table 2). The isolations were from various animal hosts which were avian (30), porcine (11), caprine/ovine (24) and bovine (4). From avian (poultry and bird), *Mycoplasma synoviae (M. synoviae)* (3), *M. gallisepticum* (3), *M. gallinarum* (9) and *M.columborale* (2) were isolated. From porcine (pig), *M. hyorhinis* (7) and *M. hyosynoviae* (1) were isolated; from caprine (goat and sheep), *Mycoplasma arginini* (17) and *M. agalactiae* (3) were isolated while from bovine (cattle), *M. bovis* (2) and *M. alkalescens* (2) were isolated. The isolation of *M. alkalescens* from cattle is reported for the first time in Malaysia. The remainder twenty-one isolates were identified only as *Mycoplasma* species due to unavailability of specific antisera.

DISCUSSION & CONCLUSION

Isolation of *Mycoplasma* from poultry showed the highest number (30), suggestive of its extent in the poultry industry in Malaysia. *M. gallisepticum* and *M. synoviae* are commonly recognized pathogens in domestic poultry birds. *M.*

gallisepticum causes disease of respiratory system in chickens and turkeys such as rhinitis and sinusitis while *M. synoviae* causes a progressive disease of synovial membrane and air sacculitis (Jordan, 1975). *M. gallisepticum* is considered as the most economically significant *Mycoplasma* pathogen of poultry and can be transmitted *in ovo* by an infected breeder to the progeny. (Levisohn and Kleven, 2000). Meanwhile, *M. gallinarum* was often regarded as non-pathogenic *Mycoplasma* (Jordan, 1979) and *M. columborale* has been isolated from pigeons (Shimuzu, 1978).

The most common species isolated from these sources was *M. arginini* which was reported to be frequently isolated from ovine with pneumonic cases (Da Massa, 1992). *M. arginini* tends to colonise rapidly, in the experimental study (Tan R.J., 1977) but *M. arginini* did not induce any lesion in goat (Goltz J.P., 1986). Another species isolated from caprine/ovine was *M. agalactiae.* This *Mycoplasma* is the main causal agent of contagious agalactiae in sheep and goats (OIE Manual, 2008).

There were eleven isolations from pigs including *M. hyorhinis* and *M. hyosynoviae*. *M. hyorhinis* is commonly found in the nasal mucosa of young piglets and under certain conditions. It was found as the only apparent pathogen causing piglet pneumonia. Isolation of *M. hyosynoviae* from swine was first reported in Malaysia (Dahlia *et al*., 2009).

Isolation of *Mycoplasma* from bovine showed the lowest number (4). *M. bovis* and *M. alkalescens* were identified from mastitis milk, both of which are on the list of major pathogens of mastitis. A number of researchers reported that *M. bovis* is an important agent of clinical mastitis in US, Australia and Europe and the infection between cows is influenced by management practices (Fillioussis *et al*., 2005). *M. alkalescens* was first isolated in Malaysia. This organism is a normal inhabitant of the upper respiratory tract of bovines and has also been associated with mastitis (Kokotovic, 2007).

More studies should be conducted on *Mycoplasma*, especially on the newly isolated species, to better understand the roles of this organism in inducing disease in their host animal.

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