ISOLATION OF *Mycoplasma iners* FROM CHICKENS

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ABSTRACT. The isolation of *Mycoplasma iners* from broiler chickens is reported for the first time in Malaysia. Chickens died after 2-3 days showing clinical signs of difficulties in movement. Post mortem finding showed cloudy air sac with chessy materials. The isolates were identified and characterized by biochemical tests. Confirmation of the isolates were carried out by growth inhibition test with specific antisera against *M. iners* (type strain PG30). The pathogenicity of the isolates was tested in chicken embryos. Results showed that *M. iners* caused 75% mortality in chicken embryos infected with 1 x 10^6 CFU/ml.

Keywords: Mycoplasma iners, chickens

INTRODUCTION

Mycoplasma iners (type strain PG30) was previously designed as serotype E. The type strain PG30 was isolated from the respiratory tract of a chicken (Barber and Fabricant, 1971). *M. iners* type strain PG30 was reported not pathogenic in chicken embryos but *M. iners* strain Oz were reported with gross and histopathological lesions in the infected chicken embryos (Wakenell *et al.,* 1995). *M. iners* is reported as first isolation in Malaysia. This paper reports the isolation of *M. iners* from chickens and its pathogenicity in chicken embryos.

MATERIALS AND METHODS

Veterinary Research Institute laboratory Ipoh received two chicken head samples from regional laboratory, Petaling Jaya for *Mycoplasma* isolation. According to the reports given by the regional laboratory, these chickens showed difficulties in movement and died after 2-3 days. Post mortem findings showed cloudy air sac with cheesy materials. Unfortunately, these samples were not received for isolation.

Sinus and trachea swabs were collected from the two chicken heads. Swab samples were cultured directly onto K-agar and K-broth (Zaini *et al*., 1992). Both media were incubated at 37°C and agar plates were incubated under 5% - 10% CO_2. After 3 days, incubation broth media showed presence of turbidity whereas mycoplasma-like colonies were detected on agar plates under a dissecting microscope at 40x magnification. Broth cultures were subcultured onto agar plates and were further incubated until mycplasma-like colonies were seen. Purification of *Mycoplasma* colonies were carried out by subculturing individual colonies of

different sizes over 3 times on agar media. 3 times cloning of the colonies were carried out by filtration method using membrane filter size 0.45 µm (Stalheim, 1976).

Five consecutive subcultures of *Mycoplasma* colonies on media without antimicrobial agents to test for reversion from an L-form to a bacterium. The purified and cloned *Mycoplasma* colonies were identified by routine and standard biochemical tests (Cottew, 1983) such as glucose fermentation, arginine hydrolysis, phosphatase activity, reduction of tetrazolium chloride and the formation of film and spots. Haemadsorbtion test using 0.3% chicken red blood cells (Sato *et al*., 1965), were carried out on the isolate. The digitonin (Sigma D5628) test (Edward, 1971) was carried out to distinguish isolates that require cholesterol for growth (*Mycoplasma* sp.) from those that do not (*Acholeplasma* sp.). Confirmation of the isolates was carried out by mean of the growth inhibition test using specific antisera on paper discs (Joseph *et al*., 1988)

Mycoplasma culture grown in K-broth was inoculated into yolk sac of 7 days embryonated SPF eggs at 1 x 10^5 CFU/ml and 1 x 10^6 CFU/ml. All eggs were incubated at 37°C. The eggs were candled daily. Dead eggs were cultured onto K-agar. Eggs that survived after 18 days were chilled for 4 hours and cultured onto K-agar.

RESULTS

Mycoplasma-like colonies were isolated from all swab samples after three days incubation on K-agar. Figure 1 showed *Mycoplasma iners* colonies after purification and cloning. Digitonin test

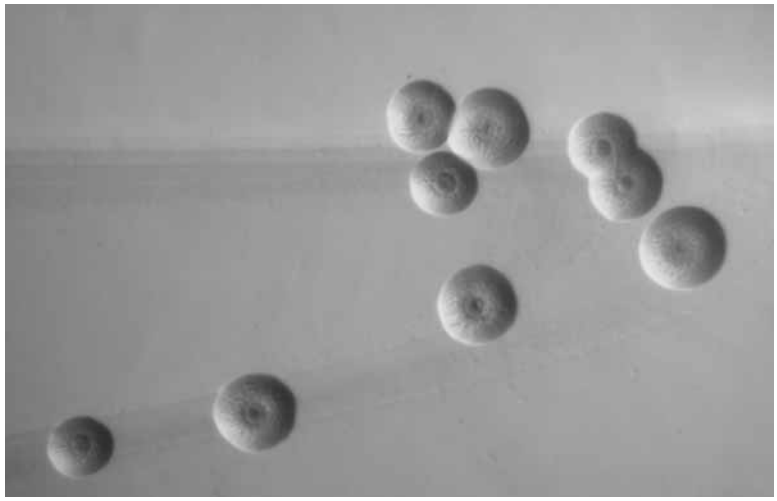

FIGURE 1. *Mycoplasma iners* colonies on K-agar after 72 hours incubation at 37°C with 5-10% CO_2 with the appearance of fried egg.

TABLE 1: Biochemical reactions of *M. iners* isolated from broiler chickens.

TESTS	RESULTS
Glucose fermentation	-
Arginine utilization	+
Formation of film and spot	+
Phosphatase activity	-
Reduction of tetrazodium chloride (TTC)	-
*Digitonin	sensitive

+ : positive - : negative

*Digitonin: sensitive indicate organism belongs to Mycoplasma sp., resistant indicate organism belongs to Acholeplasma sp.

showed sensitive result but the colonies did not have the ability to adsorb chicken red blood cells in the haemadsorbtion test. The isolate did not ferment glucose but utilize arginine, positive for the formation of film and spots, no reduction of tetrazolium chloride (TTC) and phosphatase activity as shown in Table 1. The biochemical reaction fixed the characteristic *M. iners* according to Holt *et al.* (1994).

Growth inhibition test carried out with antisera against *M. gallinarum* (type strain PG16), *M. meleagridis* (type strain 17529) and *M. iners* (type strain PG30) confirmed the isolate to be *M. iners* with a wide zone of inhibition on agar (18mm in diameter).

Embryonated SPF chicken eggs inoculated with 1 x 10^6 CFU/ml of the isolates caused 75% mortality rate on day 12 post infection whereas with 1 x 10^5 CFU/ml, a 25% mortality on day 13 post infection and 50% mortality on day 18 post infection. Reisolation of the isolate for live and dead eggs were recovered from yolk samples. Isolation from air sac swabs of the 18 days old post infection embryos were not recovered. The dead embryos at 12 and 13 days post infection were small in size. The 18 days old dead embryos were stunted in growth with the feet curve in whereas infected live embryo had curve out feet. Infected embryos showed pale liver.

DISCUSSION

In a report by Zaini *et al.* (2006), 4 species of avian *Mycoplasma* were reported for the first time in Malaysia. There were *M. columborale*, *M. gallopavonis*, *M. maculosum* and *M. pullorum*. The 2 species of *M. gallopavonis* and *M. pullorum* were reported not pathogenic to chickens embryos and SPF chickens. In the report by Wakenell *et al.* (1995), *M. iners* strain Oz showed gross histopathological lesions in chicken embryos but not in those infected with *M. iners* strain PG30. This paper described *M. iners* as the first isolation from broiler chickens in Malaysia, showing high mortality rates at 12-days-old chicken embryos. The probability of clinical signs

and pathological lesions reported in the infected chickens to be caused by *M. iners* is yet to be determined. Pathogenicity study in chickens is yet to be carried out.

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