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ISOLATION OF *MYCOPLASMA GALLOPAVONIS* FROM CHICKENS

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SUMMARY: The isolation of *Mycoplasma gallopavonis* from chickens is reported for the first time in Malaysia. The isolates were identified and characterized by biochemical and serological methods. The pathogenicity of these isolates was tested in embryonated eggs and chickens. Results showed that *M. gallopavonis* did not cause mortality to embryonated eggs and did not cause any clinical signs or gross lesions in chickens.

Keywords: *M. gallopavonis*, chickens

INTRODUCTION

Mycoplasma gallopavonis (previously designated as serotype F) was originally isolated by Roberts (1963) from air sac lesions in adult turkeys and are exclusive only to turkeys (Bencina *et al.*, 1988). However, information concerning their pathogenicity in chickens is lacking.

Mycoplasma gallopavonis resembles *M. gallisepticum*, *M. columborale*, *M. pullorum* and *M. gallinaceum* in their biochemical properties. These similarities could therefore create confusion during identification procedures.

In Malaysia, *M. gallopavonis* has not been reported, probably because there is a paucity of reports concerning diseases of turkeys which in turn is probably due to the small number of turkeys in the country. This paper reports the isolation of *M. gallopavonis* from chickens and its pathogenicity in embryonated eggs and chickens.

MATERIALS AND METHODS

Oropharyngeal swabs and blood samples were randomly collected from healthy chickens from a layer farm in Tanjung Rambutan, Perak. These swabs were immediately inoculated in K-broth (Kishima, 1990). Cultures with evidence of growth were streaked onto K-agar and incubated at 37°C in 10% CO_2 atmosphere. The isolates were identified by routine and standard biochemical tests (Cottew, 1983), indirect immunoperoxidase test (Imada *et al.*, 1987) and confirmed by growth inhibition test using specific antisera on paper discs (Joseph *et al.*, 1988). Rapid plate agglutination tests using stained *M. gallisepticum* and *M. synoviae* (Intervet, Holland) antigen were carried out on the sera collected.

Bacterial culture grown in K-Broth was inoculated into the yolk sac of 10-day-old embryonated specific-pathogen-free (SPF) eggs and incubated at 37°C. On day 10 post-infection, the eggs were cultured for re-isolation of mycoplasma. Six-week-old SPF chickens free from *M. gallisepticum* and *M. synoviae* were divided into three groups of five birds each. Group 1 birds were inoculated through the air sac with 0.5 ml of 10^7 CFU/ml of the mycoplasma culture. Group II was inoculated with double the volume through the same route while group III was inoculated with 0.2 ml of the inoculum through the nasal cavity while control chicks were inoculated through the air sac with 0.5 ml of phosphate buffered saline, pH 7.0). The four groups were housed in separate cages. The chickens were observed daily for clinical signs and at 8 days post infection, they were bled and necropsied for gross lesion examination and the tracheas and air sacs were collected and cultured for mycoplasma.

RESULTS

Isolation and Identification of Mycoplasma Isolate

Tiny fried-egg like colonies (characteristic of *Mycoplasma spp.*) grew after 3 days incubation on K-agar. Out of 20 swab samples collected, seven have the same colony appearance and biochemical characteristics (Table 1).

Table 1. Biochemical test results of isolates

Test	Result
Glucose fermentation	+
Arginine utilisation	-
Film and spot	-
Phosphatase	-
Tetrazolium choloride	-
Digitoxin	sensitive

Indirect immunoperoxidase test carried out with antisera against *M. anatis*, *M. gallisepticum*, *M. gallinaceum* and *M. gallopavonis* confirmed the isolate to be *M. gallopavonis*.

Pathogenicity in Eggs and Chickens

Embryonated eggs inoculated with 1.2 x 10^7 CFU/ml of *M. gallopavonis* did not cause any mortality to any of the eggs with no re-isolation of the bacteria.

All the chickens in the four groups including the controls did not exhibit any clinical signs and no gross lesions were observed in the respiratory tract, air sac as well as other organs in any of the chickens. *M. gallopavonis* could not be re-isolated.

DISCUSSION

In a report by Joseph *et al.* (1988), only three species of avian mycoplasma were isolated from chickens in Malaysia, i.e., *M. gallisepticum*, *M. gallinaceum* and *M. gallinarum*. In this study, *M. gallopavonis*, which was isolated from chickens is probably the first isolation in Malaysia. *M. gallopavonis*, which has similar morphology and biochemical characteristics as *M. gallisepticum* could be mistakenly identified as the

latter. It is therefore necessary to employ various serological procedures to identify a given isolate of avian mycoplasma specifically.

According to Bencina *et al.*, (1988), *M. gallopavonis* is host specific and causes air sacculitis in turkeys. *M. gallopavonis*, however, was not pathogenic to 10-day-old chicken embryos and to six-week-old SPF chickens. Therefore, it is important to take preventive measures by not having turkeys in the vicinity where chickens are harbouring *M. gallopavonis*. Unlike *M. gallisepticum* which loses its pathogenicity after several passages in culture medium, *M. gallopavonis* failed to elicit pathogenicity in chickens even though a fresh isolate was used. Although *M. gallopavonis* may not be a primary pathogen in chickens, its significance as a secondary invader is yet to be determined.

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RINGKASAN

PENGASINGAN MYCOPLASMA GALLOPAVONIS DARIPADA AYAM

Pengasingan Mycoplasma gallopavonis *daripada ayam ini merupakan yang pertama kali dilaporkan di Malaysia. Isolat ini telah dikenalpasti dan dicirikan melalui kaedah biokimia dan serologi. Kepatogenan isolat tersebut telah diuji dalam telur ayam bernas dan ayam. Hasil ujian menunjukkan bahawa* M. gallopavonis *tidak mengakibatkan kematian pada telur bernas dan tidak menyebabkan sebarang petanda klinikal atau lesi kasar pada ayam.*