Characterization of *Mycoplasma gallisepticum* strains isolated from Chickens in Malaysia

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Introduction

Mycoplasma gallisepticum (Mg) infection in poultry can lead to significant economic loss due to respiratory diseases, increased mortality, reduced growth rate and increased carcass condemnation in broiler chickens. The disease can also cause reduced egg production in laying hens and reduced hatchability in breeder birds. Proper control and preventive programs, medication of infected flock and additional labour a veterinary consultations further increase the cost of production. The disease is known to occur in Malaysia but no in-depth study was ever carried out. Many farmers have resorted to vaccinating their flock with vaccines, including live vaccine, developed in other countries and also adding antibiotics in the chicken feed. Both methods carry their own risk.

It is important to have a baseline data on the serological prevalence of the disease in Malaysia. Antigenic variations within the mycoplasma species is a well-known characteristic. It is thus also very important to identify and characterize the local pathogenic and/or predominant strains of Mg in our local poultry flocks. These two information are very critical if an effective control program is to be instituted.

Molecular biology techniques, such as DNA and protein profiling and PCR, have become increasingly popular as a diagnostic tool also, for studying the epidemiology of Mg infections in chickens. It is hoped that these techniques can be adopted and optimized for diagnostic purposes.

Materials and Methods

The project is divided into 3 parts. The first part is to establish a baseline data on mycoplasma infections in Malaysian poultry farms. The second part is to isolate and identify Mg. The third part is to characterize the predominant *M.gallisepticum* strain found in local poultry farms.

1. The seroepidemiology of *M. gallisepticum* in Malaysian poultry farms.
   (This part of the study mainly involves the examination of serum samples for antibody titers to *M. gallisepticum*. The serological techniques used will be the Rapid Serum Agglutination Test, The Haemagglutination Inhibition Test and the ELISA test).
2. Isolation and identification of *M. gallisepticum* (Samples from live and dead chickens will be cultured on PPLO medium and incubated in the CO2 incubator. Colonies suspected to be *M. gallisepticum* will be identified using the growth and metabolic inhibition tests)
3. Molecular characterization of local strains of *M. gallisepticum* and the application of PCR technique for diagnosis of Mg infection. (Both the DNA and Protein fingerprinting techniques will be used in characterizing the local strains)

Results and Discussion

Serology using the Rapid Serum Agglutination (RSA and the ELISA methods were carried out on the Broiler and Layer flocks. Seven broiler and 5 layer farms were sampled and birds were sampled randomly. A total of 822 serum samples from the broiler farms and 579 samples from the layer farms were tested using the RSA. A total of 292 serum samples from broiler farms and 291 sample from the layer farms were tested using the ELISA method. 7.5% of the serum samples from the broiler farms tested positive for Mg antibody while 32.5% of samples from the layer farms tested positive for Mg antibody by the RSA method. By the ELISA method, 13.4% and 35.4% were positive for Mg antibodies from the broiler and layer farms respectively.

Mycoplasma gallisepticum infection in chicken is a chronic respiratory infection affecting older birds mainly. It is thus expected that the seroprevalence of Mg antibodies is higher in the layer farms than the broiler farms. The presence of antibody against Mg in broiler birds is probably from maternal antibodies. The higher percentage of positive cases by the ELISA test on the broiler birds is expected as the test is more sensitive than the RSA test. However, the percentage of positive samples by the RSA and ELISA tests on the layers are similar. This probably reflects a true infection rate.

Twenty four Mg strains were isolated from 5 layer farms only. No molecular characterization were carried out then as the 3 year period of the research project ran out. However, a masters student from a different research project, under my co-supervision, is carrying the characterization work and a thesis is expected by the end of this semester (May 2003/2004).

The PCR technique was adopted for rapid diagnosis of Mg infection. 118 trachael and choanal swabs were collected from apparently healthy chicken. The primers used were from published data. Nineteen samples (16 %) were found to be positive

for Mg in which a single band of 180 bp were observed. The PCR technique can be completed in 2-3 days and is definitely faster compared to the conventional isolation and identification methods which may take up to 2 weeks to get a diagnosis. The PCR technique is also very sensitive and specific and can thus eliminate doubtful diagnosis. It is interesting to note that apparently healthy chickens carry Mg in their respiratory tracts. Whether these strains are less pathogenic or non pathogenic or the chickens are resistant to Mg infections remains to be studied. If the strains are of the "non pathogenic type", they would certainly a potential candidate to be made into live vaccine.

Conclusions

Mycoplasma gallisepticum infection is widespread in both broiler and layer chickens as shown by serology. This ia an important baseline data.

The ELISA test is more sensitive for testing broiler chickens for Mg antibodies compared to the RSA test.

The PCR technique is a better method to diagnose Mg infection in chickens compared to the conventional isolation and identification methods.

Apparently healthy chickens are carriers of Mg in their respiratory tracts.

Benefits from the study

Baseline data on Mg infection in chickens was established.

The PCR technique has now been adopted for routine diagnosis of Mg infection in chickens at the Bacteriology Laboratory of the Faculty of Veterinary Medicine, UPM.

The successful isolation and identification of 24 strains of Mg has enabled further characterization studies to be carried out to determine the molecular epidemiology of Mg in Malaysia.

Healthy chickens has been established as carriers of Mg in their respiratory tracts.

Patent(s), if applicable :

Nil

Stage of Commercialization, if applicable :

Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

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