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SEROLOGICAL AND BACTERIOLOGICAL OBSERVATIONS OF CHICKENS WITH COMPLICATED CORYZA

M.Z. Zaini and L.J. Tan

Veterinary Research Institute,
59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia

SUMMARY

The effects of mixed infection with *Haemophilus paragallinarum* and *Mycoplasma gallisepticum* on the serology and bacterial re-isolation in chickens with complicated coryza were studied. Six-week-old specific pathogen-free chickens were infected either with *H. paragallinarum* or *M. gallisepticum* alone or with a combination of both pathogens. Control chickens were not inoculated. Test chickens were re-infected with *H. paragallinarum* three weeks later and monitored at weekly intervals for five additional weeks for clinical signs, recovery of the pathogens and antibody response. All birds were then culled and examined for gross lesions. Results showed that more chickens exposed to mixed pathogens prior to the second *H. paragallinarum* infection had signs of coryza than those pre-exposed to a single pathogen ($p < 0.05$). Mixed infection prolonged the antibody response against *H. paragallinarum* infection as well as *M. gallisepticum.* Prior infection with *H. paragallinarum* or mixed pathogens significantly increased the recovery of *H. paragallinarum* ($p < 0.05$) but had no effect on the recovery of *M. gallisepticum* from the re-infected chickens. Post-mortem examination revealed that most of the infected chickens had gross lesions in the lower respiratory tract although there was no recovery of the bacteria.

Keywords: *Mycoplasma gallisepticum, Haemophilus paragallinarum,* mixed infection.

INTRODUCTION

Diagnosis of avian respiratory diseases caused by bacteria becomes complicated when more than one pathogens are involved. *Haemophilus paragallinarum* and *Mycoplasma gallisepticum* are two well-known respiratory pathogens in chickens that cause infectious coryza and avian mycoplasmosis, respectively. In countries such as Japan (Uchida *et al.,* 1985; 1990) and Malaysia (Zaini, unpublished findings), these two pathogens have been commonly isolated together as mixed infection from chickens with respiratory diseases.

Serological responses and recovery of chickens with with uncomplicated coryza have been reported by Sato and Shifrine (1964) and Yamamoto and Somersett (1964). The effects of mixed infection by *H. paragallinarum* and *M. gallisepticum* in chickens were reported by Kato (1965) and Kuniyasu *et al.* (1967). However, there are no reports on bacterial recovery and serology of chickens with mixed infection that were re-infected with coryza. The aim of this study is to determine the serological and bacterial recovery pattern of chickens with complicated coryza produced by a mixed infection with *H. paragallinarum* and *M. gallisepticum.*

MATERIALS AND METHODS

Bacteria

H. paragallinarum strain 221 of serotype A and *M. gallisepticum* strain PG 31 are reference strains obtained from the National Institute of Animal Health, Japan. These strains were kept stored at -20°C and thawed a week prior to the experiment to determine their viable counts. On the day of the experiment, the bacteria were thawed and diluted to the required inoculum dose with physiological saline.

Media

M. gallisepticum was grown for 48 h in mycoplasma broth as described by Bradbury (1977) except that the broth was supplemented with 7.5% horse serum instead of 15% swine serum. *H. paragallinarum* was cultured overnight in chicken meat infusion (CMI) broth with 10% chicken senun (Yamaguchi *et al.,* 1989). To isolate *M. gallisepticum,* 1.5% of Noble agar (Difco Laboratories, USA) was added to the mycoplasma broth without phenol-red and to isolate *H. paragallinarum,* 1.5% of Bacto agar (Difco Laboratories, USA) was added to the CMI broth. All samples collected on swabs were first streaked onto CMI agar for isolation of *H. paragallinarum* prior to mycoplasma isolation. All plates were incubated at 37°C in 5-10% $C0_2$.

Chickens

Thirty, six-week-old specific-pathogen-free chickens were randomly divided into three test groups of eight chickens and a control group of six chickens. They were reared in pressure negative isolators and fed *ad libitum.* Prior to the experiment, all chickens were bled and their sera tested for the presence of antibodies against *M. gallisepticum* and *H.*

paragallinarum serotypes A and C by the rapid serum agglutination (RSA) test and haemagglutination-inhibition (HI) test respectively. They were free from both *H. paragallinarum* and *M. gallisepticum* infections before they were used in the trial.

Rapid plate agglutination test

A stained antigen for the rapid serological detection of *M. gallisepticum* was prepared according to the method of Sato (1976) except that the organisms were cultured in mycoplasma broth instead of Frey's medium and were initially grown for 24 h instead of 3 days.

Haemagglutination inhibition test

The HI antigen was prepared according to the methods of Sawata *et al.*, (1982).

Experimental protocol

Chickens in group 1 were inoculated intranasally with 5.0 x 10^8 colony forming units (cfu) of *H. paragallinarum* while those in group 2 were inoculated intratracheally with 3.0 x 10^8 cfu of *M. gallisepticum*. Group 3 was inoculated with both pathogens using the same route and doses. The control group was left uninfected. Three weeks post-infection, all chickens in the three infected groups were superinfected intranasally with 5 x 10^8 cfu of *H. paragallinarum* and observed daily for clinical signs of rales, coughing, nasal discharge and swollen face. At weekly intervals, all birds were bled and the choanal cleft and oropharyx were swabbed.

Serum samples were subjected to the *H. paragallinarum* HI and *M. gallisepticum* RPA tests. The swabs were cultured for bacterial isolation Five weeks after the superinfection, all chickens were sacrificed before tracheal and sinus swabs were collected for bacterial isolation.

Statistical analysis

The student t-test was performed on all data using a computer program (Microsoft Excel). Significance was determined at the $p < 0.05$ level.

RESULTS

Clinical signs

Throughout the experiment, control chickens remained healthy with no signs of respiratory disease. However, chickens infected with *H. paragallinarum* alone and those received mixed infection with *M gallisepticum* showed clinical signs of respiratory infection. The group infected with *H. paragallinarum* alone showed slight degree of swollen face and clear watery nasal discharge. The mixed infection group had rales, swollen face and nasal discharge within the first three weeks of infection (Fig. 1). The number of clinically ill chickens was higher in the mixed infection group than in the *H. paragallinarum* infected group ($p < 0.05$). After re-infection with *H. paragallinarum,* all chickens in the infected groups succumbed to the infection with higher number of chickens in the mixed infection group showed clinical signs ($p < 0.05$). The clinical signs manifested by the mixed infection group were the most severe while the birds in the *M. gallisepticum* infected group were the least severely affected. The clinical signs persisted in the mixed infection group throughout the experimental period while the clinical signs in other groups became undetectable after week eight.

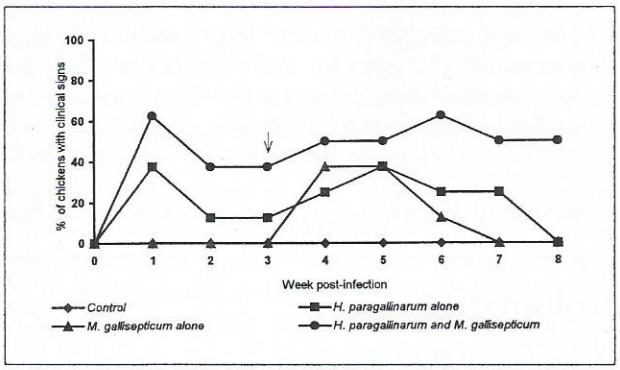

Fig. 1. Percentage of chickens with single or mixed infection showing respiratory signs. Arrow indicates the time of re-infection with *H. paragallinarum*.

Serological findings

Antibodies against *H. paragallinarum* were detected in chickens with *H. paragallinarum* alone and mixed infection as early as the first week, with higher numbers ($p < 0.05$) of serologically positive chickens in the mixed infection group (Fig. 2). Following challenge with *H. paragallinarum,* a higher percentage of chickens in the mixed infection group showed persisting antibodies while antibodies in other infected groups were not detected after five weeks.

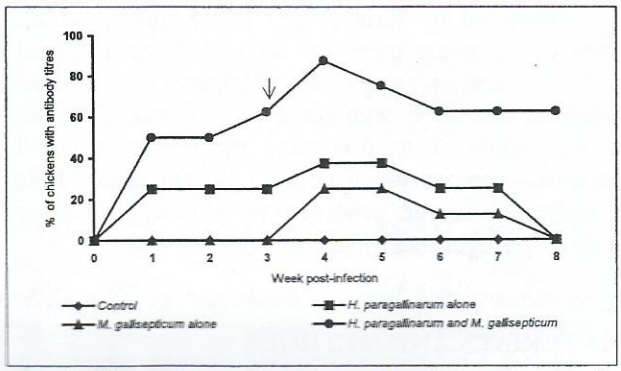

Fig. 2. Percentage of chickens showing HI antibody response against *H. paragallinarum* after single or mixed infection. Arrow indicates the time of re-infection with *H. paragallinarum*.

Rapid serum agglutination antibody response against *M. gallisepticum* infection were detected in the *M. gallisepticum* infected group and the mixed infection group within the first week of the infection and persisted after the challenge (Fig. 3). However, there was no difference in the number of serologically positive chickens between these two groups before and after the challenge.

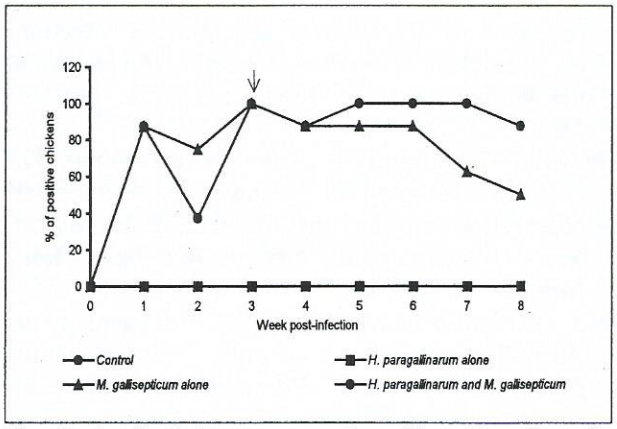

Fig. 3. Antibody response against *M. gallisepticum* in chickens infected with single or mixed infection. Arrow indicates the time of re-infection with *H. paragallinarum*.

Recovery of H. paragallinarum *and* M. gallisepticwn

H. paragallinarum was recovered from chickens in the *H. paragallinarum* infected group and the mixed infection group within the first two weeks of infection (Fig. 4). After the challenge, *H. paragallinarum* was re-isolated from the *M. gallisepticum* group but the number of positive chickens was lower than those from the *H. paragallinarum* infected group and the mixed infection group ($p < 0.05$).

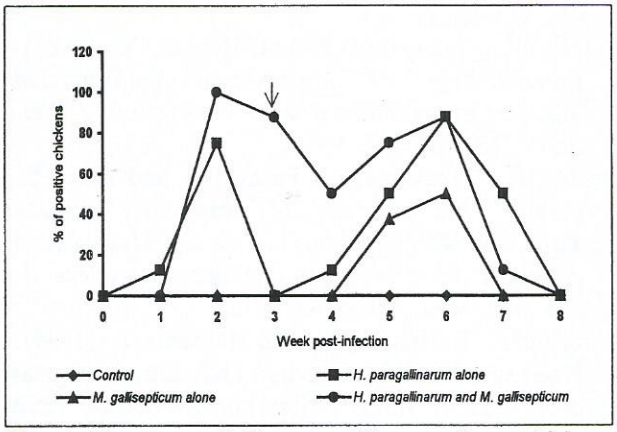

Fig. 4. Recovery of *H. paragallinarum* from chickens infected with single or mixed infection. Arrow indicates the time of re-infection with *H. paragallinarum*.

The pattern of *M. gallisepticum* recovered from the mixed infection group and the *M gallisepticum* infected group was relatively similar (Fig. 5). The percentage of *M. gallisepticum* positive chickens from both groups declined following challenge with *H. paragallinarum*. Challenging chickens with *H. paragallinarum* did not seem to affect the recovery of *M. gallisepticum*.

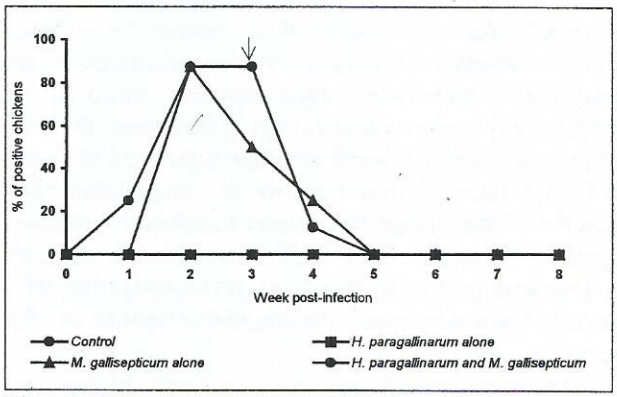

Fig. 5. Recovery of *M. gallisepticum* from chickens infected with single or mixed infection. Arrow indicates the time of re-infection with *H. paragallinarum*.

Necropsy findings

Although all infected groups were physically healthy at the end of the experiment, necropsy examination revealed that many of the infected chickens had cloudy and thickened air sacs with haemorrhages on the tracheal walls (Table 1).

Table 1. Gross lesions observed at necropsy

Group	Cloudy air sac	Thickened air sac	Haemorr trachea	Sinus exudate
Control	0/6[a]	0/6	0/6	0/6
H. para	1/8	1/8	1/8	0/8
M. galli	4/8	6/8	2/8	0/8
H. para + *M. galli*	6/8	4/8	2/8	0/8

DISCUSSION

This study showed that mixed infection with *H. paragallinarum* and *M. gallisepticum* in chickens produced severe symptoms of coryza. The initial exposure of chickens to infection with mixed pathogens seemed to influence the duration and severity of clinical manifestation. This finding is in agreement with the report by Kato (1965) that mixed infection in chickens manifests more severe symptoms of coryza that results in a more prolonged

course of disease than in birds infected with *H. paragallinarum* alone. Adler and Yamamoto (1956) characterised this type of coryza as type III when there is rapid onset and prolonged clinical signs.

Besides clinical signs, antibodies against *H. paragallinarum* also persisted when chickens were infected with mixed pathogens prior to *H. paragallinarum* infection. This shows that the immune responses against *H. paragallinarum* is longer in chickens with mixed infection compared to chickens exposed twice to *H. paragallinarum*. On the other hand, the *M. gallisepticum* infected chickens had low immune response to *H. paragallinarum* infection and prolonged duration of antibody response against *M. gallisepticum*. This observation is similar to the findings of Kuniyasu *et al.* (1967) that *M. gallisepticum* suppressed the immune response of *H. paragallinarum*.

The recovery of mycoplasma was higher in chickens that were exposed to mixed and double infection with *H. paragallinarum*. This could be attributed to the inoculum dose of *H. paragallinarum* used to infect the chickens. Recovery of mycoplasma was not enhanced by *H. paragallinarum* infection. It is postulated that being a faster grower than mycoplasma, *H. paragallinarum* is able to colonise the mucosal surface of choanal cleft and oropharyxn and possibly compete for the site of infection with mycoplasma.

Lesions in the lower respiratory tract observed at necropsy reflects the synergistic effect of *H. paragallinarum* and *M. gallisepticum* since uncomplicated coryza is limited only to the upper respiratory tract (Roberts *et al.*, 1964; Reid and Blackall, 1984). The mycoplasma damaged the defence mechanism of the upper tracts allowing the haemophilus to penetrate the lower tract and air sacs. However, the pathogens could not be recovered although gross lesions were observed. Yamamoto and Somersett (1964) observed similar lesions, which persisted for months in carrier chickens without organisms in their tissues.

Kato (1965) showed a positive correlation between clinical findings and the results of bacteriological and serological examinations. However, in this study, correlation was observed only between serological response and clinical symptoms, not with bacterial recovery.

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RINGKASAN

PENCERAPAN SEROLOGI DAN BAKTERIOLOGI PADA AYAM MENGIDAP KORIZA RUMIT

Kesan jangkitan bercampur Haemophilus paragallinarum *dan* Mycoplasma gallipsepticum *terhadap pemencilan semula serologi dan bakteria dalam ayam mengidap koriza rumit telah dikaji. Ayam bebas patogen khusus berumur enam minggu telah dijangkitkan sama ada dengan* H. paragallinarum *sahaja atau* M. gallisepticum *sahaja atau dengan campuran dua patogen ini. Ayam kawalan tidak diinokulat. Ayam ujian dijangkit semula dengan* H. paragallinarum *tiga minggu kemudian, dan dimantau setiap minggu selama lima minggu untuk petanda klinikal, gerak balas antibodi dan perolehan semula patogen. Kesemua ayam ini ditakai dan diperiksa untuk lesi kasar. Hasil kajian menunjukkan lebih banyak ayam yang didedahkan kepada patogen campuran sebelum dijangkit dengan* H. paragallinarum *kedua menunjukkan petanda koriza daripada ayam pra-terdedah kepada satu patogen sahaja* ($P < 0.05$). *Jangkitan campuran memanjangkan gerak balas antibodi terhadap jangkitan* H. paragallinarum *dan juga* M. gallisepticum. *Jangkitan dengan* H. paragallinarum *atau patogen bercampur yang dilakukan terlebih dahulu secara tererti telah meningkatkan perolehan semula* H. paragallinarum ($P < 0.05$), *tetapi tidak memberi sebarang kesan terhadap perolehan semula* M. gallisepticum *daripada ayam terjangkit semula. Pemeriksaan post-mortem menunjukkan kebanyakan daripada ayam terjangkit ada lesi kasar pada trakus pernafasan bawah, walaupun perolehan semula bakteria tidak berlaku.*