Avian mycoplasmosis in Asia

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Summary: Since 1954, avian mycoplasmosis has been considered a significant problem in chicken flocks in Japan and in other Asian countries. In Japan, Mycoplasma gallisepticum (MG) and M. synoviae (MS) infections were confirmed aetiologically in chicken flocks affected with respiratory disease or synovitis in 1962 and 1973, respectively. In other Asian countries, including Indonesia, the People’s Republic of China, Korea, Malaysia, the Philippines, Taipei China and Thailand, the occurrence of mycoplasmosis in chicken flocks has been recognised serologically or aetiologically.

Adverse atmospheric and environmental conditions, in addition to mixed infections of bacterial or viral origin, play an important role in the spread of MG and MS within chicken flocks or in the induction of clinical respiratory mycoplasmosis. Serological tests are important in determining and monitoring the mycoplasmal infection status of chicken flocks.

The establishment of mycoplasma-free breeding stocks is recognised as essential for the control of avian mycoplasmosis. To eliminate the transmission of MG to the egg, treatment of infected breeder flocks or their progeny with anti-mycoplasmal antibiotics was effective in considerably reducing the infection rate but not in entirely eliminating MG infection. The preincubation heat treatment of chicken hatching eggs has proved an effective procedure for establishing MG- and MS-free breeding stocks in Japan. Vaccination against MG infection has been practised successfully in Japan and other countries.


INTRODUCTION

Mycoplasma gallisepticum (MG) and M. synoviae (MS) infections are common diseases of chickens, not only in Asian countries. MG infections commonly cause chronic respiratory disease (CRD) of chickens, which is frequently accompanied by airsacculitis, and infectious sinusitis of turkeys (57). MS was noticed initially as an agent of synovitis, then called infectious synovitis, in broilers. However, MS was later frequently isolated from airsacculitis lesions in broiler flocks which were free from MG infections. Thus, in recent years, more interest has been shown in the role played by MS in airsacculitis than in the original infectious synovitis (30, 57).

Although a number of papers have been published on avian mycoplasmosis in Asia, most of these are presented in Asian languages. The author referred mainly to Japanese

papers in regard to the incidence of avian mycoplasmosis in Asia, transmission of infection, mixed infection and the diagnosis and control of $MG$ and $MS$ infections in chickens.

**INCIDENCE OF AVIAN MYCOPLASMOSIS**

**Situation in Japan**

Since 1954, the presence of an endemic disease, identified clinically and pathologically as CRD, has been observed principally on large-scale layer farms with chickens of multiple ages. The disease was confirmed aetiologically as respiratory $MG$ infection in 1962 (33). The first case of synovitis caused by $MG$ was observed in Oita prefecture in 1965 (35). An outbreak of keratoconjunctivitis in a young layer flock (in which morbidity reached 27.8%), apparently caused by $MG$, was reported in 1995 (27). During the period 1979 to 1985, a total of 280 $MG$ strains were isolated from 51 commercial layer farms in various geographical areas of Japan. Of 55 chicken flocks investigated, 45 showed respiratory symptoms and 10 were affected with synovitis (45).

$MS$ was first isolated from a joint and liver obtained from a chicken processing plant in the Kyushu area in 1971. Endemic respiratory infection associated with $MS$ was recognised on chicken farms in 1993. Mixed infection with $MG$ and $MS$ was also commonly observed on those farms (30, 31). The occurrence of synovitis due to $MS$ was very rare; the first case was detected in 1981 (49).

In nation-wide serological surveys conducted on selected chicken breeding flocks, positive reaction rates to $MG$ and $MS$ were as high as 55% to 74% during 1973 and 1982. However, these results were reduced to less than 20% in 1983 and 1987. The results showed that the eradication of $MG$ and $MS$ in breeding flocks has been progressing (4, 42).

However, the incidence of $MG$ and $MS$ infection as detected by serological surveys was approximately 40% in commercial layer flocks during 1984 and 1986, and approximately 10% to 20% in broiler flocks during 1980 and 1984 (45, 54).

In Japan, farms which raise turkeys are very rare. This is one of the principal reasons why research has not been conducted on mycoplasma infection among turkeys. In 1980, however, $M$. meleagridis was isolated from eight cases of airsacculitis on turkey farms located in Miyazaki (in the southern part of Kyushu) and in the Tokyo area (37).

**Situation in other Asian countries**

Complicated CRD is a disease of major importance in most Asian countries. It is commonly encountered as a result of vaccination and other stress factors. Serological surveys to detect $MG$ infections have been commonly performed. In Indonesia, seven breeding farms displayed a range of positive test result rates, from 0% to 74%. The spread of $MG$ infection was also reported in nation-wide surveys conducted on 48 layer farms, between 1981 and 1985 (29). $MG$ infection was detected serologically in Tegal ducks in west and central Java. This strain of $MG$ was initially isolated from a Tegal duck which had died of pasteurellosis (39).

In Korea, $MG$ infection in chicken flocks has been recognised since 1967, through serological surveys and isolation of $MG$ from naturally occurring cases of the disease.
In 1977, the incidence of MG infection among 20 breeder flocks from six regions of Korea was reported as ranging from 20.0% to 67.0%, averaging at 47.4% (10). Outbreaks of MS infection in broiler chicks were reported in 1979 (21).

In Malaysia, a study conducted in 1976 indicated that CRD was common, with more than 50% of 224 poultry farms affected. A serological survey on the prevalence of MG was conducted on 79 poultry farms from six selected states in Malaysia, from 1987 to 1989. An incidence of positive sera against MG of 17.2% was found. Serological evidence of MS infection was first reported in 1984, when two farms in Johore were found to have a high rate of positive sera among their flocks. Since then, serological evidence of MS infection has been detected on poultry farms in other states (58). From the first isolation of a mycoplasma (unspecified) in 1960 until 1991, only 72 of a total of 236 isolates from chickens, including 29 isolates from embryonated eggs and egg-adapted live vaccines against Newcastle disease (ND), were demonstrated to be strains of MG (8). This was as a result of the routine subjection of all egg-adapted vaccines to mycoplasma detection. The first isolation of MS from a layer flock was reported in 1992 (52).

In the Philippines, approximately 75,000 cases of CRD were reported to have occurred in the official yearly report of the Bureau of Animal Industry in 1992 (3). MG and MS infections in chickens were reported from Ruson Island in 1958 and 1971, respectively. In 1977, a serological survey was conducted on Palawan Island on 156 native chickens from 9 farms. The percentage of chickens showing positive results for antibodies to MG and MS was 35.9% and 29.5%, respectively (47).

In Taipei China, the epidemic status of MG infection has been recognised through serological surveys since 1966. In 1985, the isolation of 57 strains of MG was reported in a mycoplasmosis survey of diseased chickens. In three years of serological surveys for MG infection (from 1990 to 1992), the yearly positive rate of infection among tested chickens was 52.7% in 1990, 81.6% in 1991 and 79% in 1992. Among the 18 flocks surveyed, 16 flocks gave positive results for MG antibodies in 1990. A total of 11 out of 12 breeder flocks surveyed in 1991 gave positive results (14).

**TRANSMISSION**

MG was transmitted from carrier chickens to cohabiting susceptible birds within one to four weeks, in floor-raised flocks where direct contact among birds occurred readily. However, the disease spread much more slowly among battery-farmed chickens. MG spread through an entire flock of about 5,000 chickens over 150 days (55). Nevertheless, transmission of MS occurred rapidly in a battery-farmed chicken flock, through the drinking water, within about 50 days (11, 20).

On a commercial broiler farm, a chicken flock derived from a breeder flock infected with MG gave a high percentage of positive results to MG antibody (73.8%), when the flock was processed. Another flock originating from an MG-free breeder flock showed a lower rate of antibody (8.5%). This indicates that infected breeder flocks transmitted MG infection to their progeny through the egg (31, 55).

Although antibodies against MG or MS can be detected as early as 90 days old in some layer flocks, these infections are generally detected at approximately 170 days old. It was observed that a reduction in egg production was initiated in layer flocks when their MG antibody-positive rates reached approximately 70% (55).
The influence of mycoplasmal infection (MG and MS) on egg production in chickens has been studied. The chickens were inoculated intranasally with both MG and MS at 2, 8 or 20 weeks of age, and their egg production was surveyed. The egg production rate of chickens infected at an older age was lower (by 4% to 6.3%) than that of chickens infected at a younger age. This result showed that the egg production rate of chickens was drastically reduced when they were infected with MG and MS at the age of sexual maturity (7).

**COMPLICATING FACTORS**

In general, chickens infected with only MG or MS produce no clinical symptoms. However, it is known that chickens infected subclinically with MG or MS can develop CRD or severe respiratory lesions in complication with other infectious agents. In the field, this phenomenon has been considered to be important, not only in relation to natural infection with various bacteria such as *Haemophilus paragallinarum*, *Escherichia coli* and viruses, but also in regard to the use of live-virus vaccines against viral respiratory diseases of chickens, such as ND and infectious bronchitis (IB) (1, 9, 25, 26, 34).

When chickens were inoculated only with MG, no clinical signs were observed and the amount of MG in the tracheal membrane decreased periodically. However, in chickens infected simultaneously with MG and ND virus B1 (NDV-B1), severe respiratory symptoms were observed and multiplication of MG in the trachea rapidly reached $10^6$ to $10^7$ approximately 3 days after inoculation, then decreased gradually (25).

In 1981, an outbreak of infectious synovitis due to MS was diagnosed in Japan for the first time. *Mycoplasma gallinaceum* (MGC) was also isolated from the joints of these chickens and, furthermore, the affected birds demonstrated positive results in serological tests for antibodies to infectious bursal disease (IBD) virus. The effects of inoculation of MGC in combination with previous exposure to IBD virus (IBDV) were investigated in relation to MS infection in chickens. The results of these studies indicate that concomitant infection of MGC acts synergistically with MS, and that previous exposure to IBDV increases the susceptibility of the synovial tissue to MS infection (49). Synovitis was also reproduced in chicks inoculated intranasally with the MG isolate when IBDV was administered orally at the same time (43).

It has been considered that adverse atmospheric conditions, as well as mixed infection, play an important role in the spread of MG or MS among chicken flocks, and on the induction of the clinical phase of respiratory mycoplasmosis. The susceptibility of chickens to MG or MS was significantly increased in the presence of a high concentration of ammonia fumes or dust, due to a reduction in normal physiological activities of the respiratory mucous membrane. The multiplication of MG in the respiratory tract of chickens exposed to 50 ~ 100 parts per million (ppm) of ammonia, was enhanced remarkably (up to $10^6$ colony-forming units [CFU] per ml), as compared with that in chickens not exposed to ammonia ($10^2$ CFU/ml). On the 28th day after exposure to ammonia, these chickens showed respiratory symptoms similar to those seen in natural CRD cases. Even in the presence of a low concentration of ammonia, such as 20 ppm, multiplication of MG in the trachea was enhanced in chickens exposed to such conditions for 7 days (36).
DIAGNOSIS

In Japan, MG- and MS-stained antigens for the rapid plate agglutination test (RPAT) were developed in 1965 and 1976, respectively (2, 12, 30). In the laboratory, tube agglutination tests (TA) and haemagglutination (HA) inhibition (HI) tests, using HA antigen produced in Japan, have been employed (1, 11, 30). The diagnostic standards for positive reactions in these serological tests are shown in Table I (2, 12, 30). In Indonesia, imported MG-stained antigen for the RPAT was used in 1977. However, an MG-stained antigen was prepared at the Research Institute for Animal Diseases (Balai Penelitian Veteriner) in Bogor (Indonesia), and breeder flocks were surveyed using a rapid serum agglutination test from 1981 to 1985 (29).

| TABLE I |

Diagnostic standards in various serological tests for Mycoplasma gallisepticum and M. synoviae infections of chickens in Japan (2, 11, 30)

<table>
<thead>
<tr>
<th>Serological test</th>
<th>Mycoplasma gallisepticum</th>
<th>Mycoplasma synoviae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Suspicious</td>
</tr>
<tr>
<td>Whole blood plate agglutination</td>
<td>&lt; 1 min</td>
<td>&gt; 2 min</td>
</tr>
<tr>
<td>Serum plate agglutination</td>
<td>&lt; 1 min</td>
<td>&gt; 2 min</td>
</tr>
<tr>
<td>Tube agglutination</td>
<td>&gt; 1:20</td>
<td>1:10</td>
</tr>
<tr>
<td>Haemagglutination inhibition</td>
<td>&gt; 1:10</td>
<td>1:5</td>
</tr>
</tbody>
</table>

A dot-immunobinding technique (dot-enzyme-linked immunosorbent assay or dot-ELISA) seems to be useful for a rapid, simple and specific diagnosis of avian mycoplasmosis. Both MG and MS antigens prepared for the routine HI test were diluted and adsorbed onto separate pieces of durapore membrane for the measurement of dot-ELISA titres of test sera. In addition, durapore strips bearing both antigens were employed for dot-ELISA with chicken sera diluted 100-fold (38). In Malaysia, ELISA and dot-immunoblot (DIB) techniques were improved in specificity for MG and MS antibodies by using antigens made from young cultures and by blocking the antigen-coated plates and diluting test serum with skim milk containing buffer (53).

Maternal antibody for MG or MS was detected in the chicks of infected breeding chickens. Although this maternal antibody decreased quickly and disappeared within two weeks of hatching, chicks should be inspected for maternal antibody before they are two days old. Generally, in chicks infected with MG or MS, agglutination antibody against the mycoplasma appeared four to six weeks after infection, although it appeared two to three weeks after infection in chickens older than two months (11, 12, 30). It is recommended that serological inspection for MG or MS infection in commercial chicken flocks is conducted on chickens aged 100 days or more, because this is the age at which mycoplasmal antibodies generally appear (12, 32).
In chicks inoculated intranasally with MS, antibody titres in the agglutination and HI tests peaked at 12 weeks old. However, the development of HI antibody is delayed when compared with that of agglutinin as recognised in chicks infected with MG, so that a positive reaction with the agglutination test and a negative reaction in the HI test may occur at an early stage after infection. The inoculated MS was isolated from respiratory tracts at 19 weeks of age, indicating a long period of existence for the organism (11, 12, 32).

When using serum RPAT, non-specific reactions occur in some flocks which are inoculated with an inactivated tissue-cultured IBDV vaccine. This is caused by cross-reaction between the antibody for serum component in the tissue culture of the IBDV vaccine and the serum factor adsorbed on the mycoplasma antigen. It has been recommended that, to avoid the cross-reaction, a drop of fresh horse or pig serum be added to the test serum in the RPAT (5). To confirm specificity of the reaction in RPAT, the HI, TA and ELISA tests were used (32).

CONTROL

Procedures for the control of MG and MS were mainly dependent on the prevention of transmission of the disease through eggs by the establishment of MG- and MS-free breeding flocks. On commercial chicken farms, this programme includes buying mycoplasma-free chicks and strict isolation of the flock to avoid the introduction of these diseases. Current control programmes are supported by regular serological monitoring of the status of flocks, while prophylactic medication of chicken flocks has also been administered during the growth period. However, medication of egg-laying flocks has been strictly limited to avoid drug residues in eggs. In recent times, MG vaccines have also been used to prevent reduction in egg production (1, 31, 57).

Procedures for the establishment of mycoplasma-free breeding flocks

Procedures have been formulated for the establishment of MG-free breeding flocks. For the selection of a breeding flock from which hatching eggs will be used to establish mycoplasma-free stock, two serological tests are performed at one-month intervals. If the antibody titres of this flock remain the same, the flock is selected and antibiotics effective for MG (such as tylosin, etc.) are administered. Such treatment prevents or reduces the production of MG-contaminated eggs for a period of approximately one month. Furthermore, if necessary, the hatching eggs are treated with antibiotics to eliminate transmission of mycoplasma, using the egg dipping method or injection into the eggs. Chicks resulting from the treated eggs are divided into small flocks (fewer than 500 chicks each) and are raised in strict isolation with regular serological monitoring. Flocks including seropositive chickens are discarded. These procedures were repeated over two generations. When complete elimination of the infection was achieved, the flocks were determined to be MG-free breeding stocks (1, 16, 19).

For the establishment of MS-free breeding flocks, the egg heating procedure, which had been developed by Yoder, seemed more promising than the egg-dipping method (56). Eggs at room temperature (24.4°C) were heated in an incubator over 13.5 h to reach a temperature of 46.6°C, to inactivate MS contamination. The temperature was
gradually raised to the critical point using a special thermal regulator developed by Murayama (17). In this method, the hatchability of heated eggs was reduced by 4% to 15% (17, 18, 19).

Treatment with antibiotics

MG and MS are highly susceptible to macrolide and tetracycline antibiotics, which have been used for the prevention or treatment of avian mycoplasmalosis (12, 44). In Japan, these antibiotics have been used to prevent the induction of clinical respiratory distress in chickens due to inoculation of live ND and IB vaccines (40). Recently, however, the efficacy of tylosin in treating chickens has been reduced, due to an increasing number of tylosin-resistant strains in the field (13). As a result, such new antibiotics as 3-acetyl-4'-isovaleryl tylosin, mirosamin and new quinolone derivatives have been developed and employed (28, 41, 46).

Vaccination

Layer chickens are raised over a long period, generally for more than 18 months. There are many large, multiple-age layer farms in egg-producing areas of Japan. Although MG-free chickens have generally been available for commercial operations, such farms have the problem of continuous reinfection with MG and MS, due to persistence of the organism in the older group of birds. Under such management conditions, MG- and MS-positive status are usually accompanied by a poor production performance for that flock. In such a situation, the commercial egg industry has tended to use live or inactivated MG vaccine to assist in controlling this problem (57). Since 1989, in Japan, oil-emulsified MG bacterin which is imported from the United States of America (USA) and a Japanese product of aluminium hydroxide-adsorbed MG bacterin (another new Japanese oil-emulsified preparation was approved in 1995) have been commonly used on layer farms (50). In 1995, a live MG ts-11 strain vaccine was imported from Australia, which was officially approved for commercial use (48).

Vaccination with these bacterins did not completely prevent MG infection. However, vaccination did accelerate the elimination of the organism, prevent clinical signs and pathological lesions, and increase egg production. The number of eggs in the hen house (formula: total number of eggs produced during inspection/number of chickens at the beginning of inspection) from flocks vaccinated with the oil-emulsified bacterin or the aluminium hydroxide-adsorbed product increased by 1 to 21 eggs, compared with production from unvaccinated flocks (15, 51).

An inactivated MG bacterin has been imported into Korea since 1986. To replace this imported bacterin, an oil emulsion bacterin was developed and field trials were conducted in 1992 (22, 23). In China, it was reported in 1994 that a formalin-inactivated oil emulsion MG bacterin had been developed, which was available for inoculation by the subcutaneous or intramuscular route in chickens and chicks. More than 20 million doses of this vaccine have been applied successfully in over 21 provinces of China (6). In 1992, there was a report on experimental studies on vaccination with live MG vaccine (F strain) (24). In 1989, it was reported that imported inactivated MG bacterins had been used in Malaysia (58).
LA MYCOPLASMOSE AVIAIRE EN ASIE. – S. Sato.


Les conditions atmosphériques et environnementales défavorables, auxquelles s’ajoute la présence d’autres affections d’origine bactérienne ou virale jouent un grand rôle dans la propagation de M. gallisepticum et de M. synoviae dans les élevages de volaille ou dans le déclenchement d’une mycoplasmose respiratoire clinique. Les épreuves sérologiques constituent un outil important pour déterminer et surveiller le statut des élevages aviaires à l’égard de la mycoplasmose.

Pour lutter contre la mycoplasmose aviaire, il est essentiel de pouvoir garantir que les élevages de reproducteurs sont exempts de mycoplasmes. En cas d’infection dans un élevage, le traitement par antibiotiques des reproducteurs ou de leur descendance permet d’empêcher la transmission de M. gallisepticum aux œufs : un tel traitement a considérablement réduit le taux d’infection, sans pour autant éliminer totalement l’infection due à M. gallisepticum. Le traitement thermique des œufs avant incubation est un procédé efficace qui a conduit à l’établissement d’élevages de reproducteurs exempts de M. gallisepticum et de M. synoviae au Japon. La vaccination contre l’infection par M. gallisepticum a été pratiquée avec succès au Japon et dans d’autres pays.


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LA MICOPLASMOSIS AVIAR EN ASIA. – S. Sato.

Resumen: Desde 1954, la micoplasmosis aviar viene siendo considerada un serio problema para la avicultura en Japón y otros países asiáticos. En Japón, se comprobó etiológicamente que Mycoplasma gallisepticum y M. synoviae eran los agentes responsables de los casos de enfermedad respiratoria crónica o de sinovitis que afectaron a los pollos en 1962 y 1973, respectivamente. En otros países asiáticos, a saber Indonesia, la República Popular de China, Corea, Malasia, Filipinas, Taipei China y Tailandia, se han detectado, por métodos serológicos o etiológicos, casos de micoplasmosis en los pollos.

Las condiciones atmosféricas y ambientales adversas, junto con infecciones asociadas de origen bacteriano o vírico, desempeñan un importante papel en la propagación de M. gallisepticum y M. synoviae en las granjas avícolas o en la aparición de micoplasmosis respiratorias clínicas. Las pruebas
serológicas son un método de gran importancia para determinar y vigilar la condición sanitaria de las granjas en cuanto a posibles infecciones micoplasmáticas.

El establecimiento de bandadas reproductoras libres de micoplasmas es una etapa crucial del control de la micoplasmosis aviar. Para eliminar la transmisión de M. gallisepticum al huevo, la aplicación de un tratamiento de antibióticos anti-micoplasmáticos a bandadas reproductoras infectadas o a su progenie se reveló un método eficaz, en la medida en que redujo considerablemente la tasa de infección, pero no llegó a eliminar por completo la infección por M. gallisepticum. En Japón, el tratamiento térmico previo de los huevos por incubar ha demostrado ser un método eficaz para establecer bandadas reproductoras libres de M. gallisepticum y M. synoviae. También se ha aplicado con éxito, en Japón y otros países, la vacunación contra la infección por M. gallisepticum.


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REFERENCES


