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Surveillance of *Mycoplasma gallisepticum* Infection in Mixed Thai Native Chickens in the Area of Nakornpathom Province

Somsak Pakpinyo[1*] Somkid Khanda[2] Thawat Lekdamrongsak[1]

Abstract

This study was to conduct a surveillance of *Mycoplasma gallisepticum* (MG) infection in mixed Thai native chickens. Samples were submitted from 30 mixed Thai native chicken flocks, 15 birds per flock, aged between 1 and 4 months without MG vaccination in the area of Nakornpathom province during the September 2005 - October 2006 period. Each bird was bled for MG serology by a serum plate agglutination (SPA) test and enzyme linked immunosorbent assay (ELISA) test kits and swabbed for MG antigen detection by polymerase chain reaction (PCR) technique. Results revealed that the positive reactors detected by the SPA test, ELISA and PCR procedure were 7, 8 and 6 flocks, respectively. There were 5 flocks that were detected to have positive reactors in all tests. The percentage of positive results depending on age: 1 month, 1-2 months, 2-3 months and 3-4 months tested by SPA, ELISA and PCR was 18.2-40%, 16.7-40% and 18.2- 40%, respectively. This study found that the older the flock the higher the number of positive reactors was found. MG DNA was determined in birds older than 1 month. In addition, respiratory clinical signs were probably observed in the MG infected flocks.

Keywords : ELISA *Mycoplasma gallisepticum* native chickens, PCR SPA,

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บทคัดย่อ

การสำรวจการติดเชื้อ *มัยโคพลาสมา กัลลิเซพติกุม* ในไก่ผสมสามสายพันธุ์ ในเขตจังหวัดนครปฐม

สมศักดิ์ ภัคภิญโญ[1*] สมคิด ขานดา[2] ธวัช เล็กดำรงศักดิ์[1]

วัตถุประสงค์ของการศึกษาเพื่อทำการสำรวจและเฝ้าระวังการติดเชื้อ *มัยโคพลาสมา กัลลิเซพติกุม* (เอ็มจี) ในไก่ผสมสามสายพันธุ์ ตัวอย่างได้รับจากฟาร์มในเขตจังหวัดนครปฐม จำนวน 30 ฝูง ๆ ละ 15 ตัว อายุระหว่าง 1-4 เดือน โดยไม่มีประวัติการทำวัคซีนเอ็มจี ระหว่างเดือนกันยายน 2548 - ตุลาคม 2549 ไก่แต่ละตัวจะถูกทำการเจาะเลือดเพื่อตรวจทางซีรัมวิทยา ด้วยวิธีเอสพีเอ และวิธีอีไลซา และถูกทำการป้ายเชื้อเพื่อตรวจหาเอ็มจีแอนติเจน ด้วยวิธีพีซีอาร์ ผลพบว่า พบผลบวกที่ตรวจด้วยวิธีเอสพีเอ อีไลซา และพีซีอาร์ จำนวน 7, 8 และ 6 ฝูง ตามลำดับ โดยพบว่า 5 ฝูงที่ให้ผลบวกต่อการตรวจทั้ง 3 วิธี หากพิจารณาตามช่วงอายุ 1 เดือน 1-2 เดือน 2-3 เดือน และ 3-4 เดือน พบผลบวกที่ตรวจด้วยวิธีเอสพีเอ อีไลซา และพีซีอาร์ ร้อยละ 18.2-40, 16.7-40 และ 18.2-40 ตามลำดับ การศึกษาครั้งนี้แสดงให้เห็นว่าฝูงไก่อายุมากขึ้น จำนวนตัวอย่างที่ให้ผลบวกพบมากขึ้นด้วย ดีเอ็นเอของเอ็มจีถูกตรวจพบที่ไก่อายุมากกว่า 1 เดือน นอกจากนี้อาการของระบบทางเดินหายใจอาจจะพบได้ภายในฝูงไก่ที่ติดเชื้อเอ็มจี

คำสำคัญ : อีไลซา *มัยโคพลาสมา กัลลิเซพติกุม* ไก่ผสมสามสายพันธุ์ พีซีอาร์ เอสพีเอ

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Introduction

Mycoplasma gallisepticum (MG) infection is known as a chronic respiratory disease (CRD) of poultry (Ley, 2003). Chickens, turkeys, quails, partridges, pheasants, pigeons, etc. are natural hosts of the MG infection (Ley, 2003). Infected birds show respiratory signs including conjunctivitis, sneaking or sneezing leading to a vaccine reaction when these birds receive a live virus vaccine. The morbidity rate is high but the mortality rate is low unless there is no secondary infection. MG transmission occurs via direct contact or horizontal transmission and from the eggs or via vertical transmission. For the vertical transmission, the newly hatching chicks show a high mortality rate and/or an unhealthy appearance.

MG infection causes economic loss in broilers including a poor feed conversion rate, and poor carcasses. In layers or breeders, a decrease in egg production of up to 21 eggs/bird, the small size of eggs, and a high rate of infertile eggs have been observed (Mohammed et al., 1987). Furthermore, the cost of treatment, prevention and control of MG infection should be considered. For these reasons, the US has brought MG control and eradication into the National Poultry Improvement Program (NPIP).

MG infection can be diagnosed by 2 main methods: antigen detection and serology. Antigen detection can be done by culture technique and DNA detection by polymerase chain reaction (PCR) technique. Culture technique is time consuming for MG growth and requires a growth inhibition or immunofluorescent test to confirm the Mycoplasma colony; however, this technique is known as the gold standard. The PCR technique is rapid, sensitive, specific and widely used in diagnostic and institutional laboratories. Serology is useful for flock monitoring in the MG control program and to aid in diagnosis when the flock is suspect. Serology procedures can be tested by serum plate agglutination (SPA), hemagglutination inhibition(HI) and enzyme-linked immunosorbent assay(ELISA).

In Thailand, some broiler and broiler breeder farms have been found with MG infection; therefore, infected farms have established a MG clean status of breeder flocks and have maintained that status by prevention, control and a biosecurity program. There have been reports of MG infection in pheasants, peafowl, chukar patridges and backyard chickens in the US (Cookson and Shivaprasad, 1994; Bencina et al., 2003). As we know, most backyard chickens or the mixed Thai native chickens are owned

by small farm holders, which usually have inadequate biosecurity that easily introduces MG organisms into the farms. However, no reports of MG infection in mixed Thai native chickens have been determined. Therefore, this study investigated the surveillance and monitoring MG status in mixed Thai native chickens. The data of this study will be useful for the growers, veterinarians and servicemen to prevent and control MG infection on farms.

The objective of this study was to investigate the surveillance of MG infection in mixed Thai native chickens by serology and PCR technique.

Materials and Methods

Samples were submitted from 30 mixed Thai native chicken flocks in the area of Nakornpathom province, 15 birds per flock, aged between 1 and 4 months and having no MG vaccination program during September 2005-October 2006 period. Most flocks were healthy in a condition, but only flock numbers 25 and 27 showed mild respiratory signs while collecting samples. Individual birds were bled at the wing vein, swabbed at the choanal cleft and then the number of blood and swab samples were identified. The blood samples were separated for MG serology. The swab samples were inoculated into 2 ml of Frey's broth medium supplemented with 15% swine serum(FMS) (Kleven and Yoder, 1989) and submitted to the laboratory for DNA detection by PCR technique.

MG serology.

SPA procedure. Fresh sera were tested with MG antigen (Nobilis®, Intervet International B.V., Holland) following the manufacturer's instructions. Briefly, thirty µl of serum were mixed with 30 µl of antigen then incubated at room temperature for 1-2 min before the result would be observed. Negative and positive sera were also included in each test.

ELISA. Sera were tested with commercial test kits, ProFLOK® (Synbiotics Corporation, USA) and followed the manufacturers' directions. Briefly, diluted sera were added into MG antigen-coated plate, incubated, washed then peroxidase-labeled anti-chicken antibody (conjugated antibody) was added. After incubation, the plate was washed then substrate was added and, finally, the stop solution was added. The plate was read in an ELISA reader manufactured by Labsystems Multiskan MS Type 352, Finland. The optical density of the negative, positive control and samples was calculated then interpreted according to the manufacturers' recommendation. For the interpretation of ProFLOK® ELISA, titer levels 0-148, 149-743, and equal or higher than 744 were negative, suspicious, and positive reactor, respectively.

DNA detection.

PCR procedure. The broth sample was individually determined in this study. This method is described by Lauerman (1998). Briefly, the broth was extracted for DNA template by centrifugation at 15,000 xg, washed with distilled water, followed by dilute pellete with distilled water, boiling for 10 min, then placing at -20°C for 10 min, ending with centrifugation and collection of the supernatant at -20°C until use. For PCR mixture in 50 µl volume, KCl 500 mM, Tris-HCl (pH 8.3) 100 mM, dNTP (Fermentas) 1 mM, primer F (5'GAGCTAATCTGTAAAGTTGGTC3') and primer R (5'GCTTCCTTGCGGTTAGCAAC3') (Qiagen) 10 pmole each, Taq polymerase (Fermentas) 1.25 U and DNA template 5 µl (250 ng). MS strain WVU 1853 (ATCC 25204) and MG strain S6 (ATCC 15302) was used as a negative and positive control, respectively. PCR mixtures were amplified in a DNA thermal cycler (PCR Sprint, Thermo Electron Corporation, Milford, MA) with 94°C for 30 sec, 55°C for 30 sec and 72°C for 60 sec for 40 cycles and followed by 72°C for 5 min. The PCR product was analyzed in 2% agarose gel (Pharmacia Biotech AB, Uppsala, Sweden), stained with ethidium bromide, visualized by UV transilluminator, and photographed.

Results

The numbers of positive flocks tested by SPA, ELISA and PCR were 7 (flock number 2, 3, 4, 8, 18, 25 and 27), 8 (flock number 1, 2, 4, 5, 15, 18, 25 and 27) and 6 flocks (flock number 2, 4, 15, 18, 25 and 27), respectively. In addition, there were 16 suspected flocks tested by ELISA (flock number 6, 7, 10, 11, 12, 13, 14, 17, 19, 21, 22, 23, 24, 26, 28 and 30). Overall, there were 5 flocks (flock number 2, 4, 18, 25 and 27) that detected the positive reactors in all tests (Table 1).

The percentage of positive results depending on age: 1 month, 1-2 months, 2-3 months and 3-4 months tested by SPA, ELISA and PCR was 18.2- 40%, 16.7-40% and 18.2- 40%, respectively (Table 2).

Table 1 Numbers of positive flocks tested by SPA, ELISA and PCR

Flock I.D.	Numbers of samples	Submitted date	Age (month)	Numbers of positive flocks/total		
				SPA	ELISA	PCR
1	15	14/9/2005	2	0/15	1/12 (3)*	0/15
2	15	21/9/2005	3.5	15/15	15/15	4/15
3	15	28/9/2005	2	3/15	0/15	0/15
4	15	28/9/2005	4	15/15	12/14 (1)	4/15
5	15	28/9/2005	1	0/15	1/15	0/15
6	15	9/11/2005	2	0/15	0/13 (2)	0/15
7	15	16/11/2005	1.7	0/15	0/14 (1)	0/15
8	15	7/12/2005	3	1/15	0/15	0/15
9	15	7/12/2005	3	0/15	0/15	0/15
10	15	7/12/2005	3	0/15	0/14 (1)	0/15
11	15	7/12/2005	3	0/15	0/12 (3)	0/15
12	15	23/12/2005	3.5	0/15	0/11 (4)	0/15
13	15	11/1/2006	2	0/15	0/14(1)	0/15
14	15	18/1/2006	2	0/15	0/14 (1)	0/15
15	15	31/3/2006	1.5	0/15	1/13 (2)	11/15
16	15	4/4/2006	2	0/15	0/15	0/15
17	15	11/4/2006	3	0/15	0/14 (1)	0/15
18	15	20/4/2006	2	6/15	3/6 (9)	5/15
19	15	20/4/2006	1	0/15	0/14 (1)	0/15
20	15	26/4/2006	1	0/15	0/15	0/15
21	15	1/5/2006	2	0/15	0/14 (1)	0/15
22	15	16/5/2006	1.5	0/15	0/9 (6)	0/15
23	15	19/7/2006	4	0/15	0/12 (3)	0/15
24	15	16/8/2006	4	0/15	0/6 (9)	0/15
25	15	30/8/2006	3	15/15	3/7 (8)	15/15
26	15	6/9/2006	1	0/15	0/14 (1)	0/15
27	15	28/9/2006	2.5	12/15	2/4 (11)	14/15
28	15	19/10/2006	3	0/15	0/14 (1)	0/15
29	15	27/10/2006	1	0/15	0/15	0/15
30	15	27/10/2006	1	0/15	0/14 (1)	0/15

*the number within parenthesis means the numbers of the suspected reactors in the flock

Table 2 Percentage of positive results depending on age: 1 month, 1-2 months, 2-3 months and 3-4 months tested by SPA, ELISA and PCR.

Age (month)	Numbers of flocks	Numbers of positive flock (%)		
		SPA	ELISA	PCR
1	6	0	1 (16.7%)	0
1 - 2	11	2 (18.2%)	3 (27.3%)	2 (18.2%)
2 - 3	8	3 (37.5%)	2 (25%)	2 (25%)
3 - 4	5	2 (40%)	2 (40%)	2 (40%)

Discussion

The mixed Thai native chickens owned by small farm holders in the Nakornpathom province area were apparently found to have antibody response and MG DNA at over 1 month old. However, ELISA could detect only 1 positive reactor and none of the suspected reactor in flock number 5 at 1 month old, whereas the SPA test showed a negative reactor. Generally, the SPA test can be used as a screening test for MG infection and generally shows positive reactors at about 7-10 days post vaccination or after inoculation, because SPA detects immunoglobulin (IgM), which is the first immunoglobulin to be formed after infection (Kleven, 1975; Kleven, 1981). For the positive reactor determined by the ELISA, this suggests that the bird had derived high maternal antibody (IgG), which remained at a detectable level at 1 month old. The SPA and PCR tests of the flock number 5 showed negative results, which indicated that this flock should be free of MG infection. Therefore, this was reason why we suggested the bird derived high maternal antibody. This suggestion may not concur to the study determined by Snell and Cullen (1977) found that the maternal antibody of the chick cannot be detected at 18 days old or older. The breed or genetic, environment, or management are possible factors which influence the declination of the maternal antibody. However, this suggestion can be confirmed by recollecting samples in 2 weeks or later. Form this reason; therefore, this study determined the surveillance of MG infection at 1 month old or older to avoid the maternal antibody result.

For the suspected flocks determined by the ELISA, 16 flocks showed the evidence of MG infection. Conversely, the SPA and PCR procedures could not detect the evidence of MG infection in these flocks. In general, the SPA procedure is slightly more sensitive than the ELISA (reviewed by Ley, 2003). Therefore, the most of suspected flocks determined by the ELISA might be the false suspected flocks, and need to be confirmed in later. Interestingly, flock number 11, 12, 22, 23 and 24 were found 3 or more suspected samples in the flock. Most of these flocks were 3 months or older, suggesting that farm management or biosecurity was not as well as that of during younger birds' life.

The incubation period of the experimentally infected with MG organisms is 6-21 days (Bradbury et al., 1994; Bradbury and Levisohn, 1996). Conversely, the incubation period of the natural infection possibly takes longer than 6-21 days. Therefore, 1 month old birds did not obviously exhibit the antibody titer, evidence of MG infection or any clinical signs. This study suggests that flocks had a high chance of infection with MG organisms after rearing the birds for 1 month or more. Interestingly, several studies also found that many older infected flocks, which are usually subclinical signs, appear to delay the onset of the first serologic response (Truscott et al., 1974; Yoder, 1986). The possibly reason is good farm management and biosecurity during the young bird's life or the brooding period compared with that of older bird rearing, leading to the low chance of MG infection in young birds.

In this study, flock number 15, MG infection could be detected by ELISA and PCR procedure in flock aged 1.5 month or older with 1 and 11 samples out of 15 samples, respectively. Interestingly, this flock revealed several samples of MG DNA detected by PCR procedure, but rarely revealed the antibody reactor detected by either SPA test or ELISA and any clinical signs, indicating the early MG infection in this flock.

Regarding sampling history, the high number of positive reactors detected by SPA, ELISA and PCR in the older flocks, the respiratory signs including coughing, sneaking, conjunctivitis and watery eyes had been observed in the flock numbers 4, 18, 25 and 27 flocks. For the flocks numbers 25 and 27 located in the previous positive farms (flocks number 4 and 18, respectively) detected by all procedures had a high chance of MG infection. This confirmed that the management and biosecurity of the positive farm were not efficient. MG organisms can be transmitted by contaminated vectors including growers, equipment, etc. Christensen et al. (1994) found that MG organisms can sustain their viability on cotton clothes, in hair or in the nasal passage for 4, 3 and 1 day, respectively. Therefore, effective farm management and biosecurity can prevent the MG infection and reduce the cost of the therapeutic treatment.

Conclusions

In surveillance of *Mycoplasma gallisepticum* infection in mixed Thai native chickens in Nakornpathom province during the September 2005 to October 2006 period, 30 flocks, aged between 1 and 4 months determined by SPA test, ELISA and PCR procedure were investigated. The positive reactors detected by the SPA test, ELISA and PCR procedure were 7, 8 and 6 flocks, respectively. This study indicates that in flocks infected with MG organisms, birds possibly showed clinical respiratory signs. In addition, the older the flock the higher number of positive reactors was found. The SPA test is appropriated to monitor the surveillance of MG infection in the suspected flocks.

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