Original Article

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

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Abstract

This study was to conduct a surveillance of *Mycoplasma synoviae* (MS) infection in mixed Thai native chickens. Samples were submitted from 30 mixed Thai native chicken flocks, 15 birds per flock, aged between 1-4.5 months in the area of Nakornpathom province in the September 2005-October 2006 period. Each bird was bled for MS serology by serum plate agglutination (SPA) and enzyme linked immunosorbent assay (ELISA) test kits and swabbed for MS antigen detection by polymerase chain reaction (PCR) technique. Results revealed that the positive reactors detected by the SPA test, ELISA and PCR procedure were 12, 9 and 8 flocks, respectively. There were 4 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 3-4.5 months tested by SPA, ELISA and PCR was 0-60%, 0-60% and 0-50%, respectively. This study found that the older the flock the higher the number of positive reactors found. MS DNA was determined in birds older than 2 months. Even so, clinical signs were not observed in the MS infected flocks in practical field.

Keywords : ELISA, mixed Thai native chickens, *Mycoplasma synoviae,* PCR, SPA

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บทคัดย่อ

การสำรวจการติดเชื้อ *มัยโคพลาสมา ซินโนวีอี*ในไก่ผสมสามสายพันธุ์ในพื้นที่จังหวัดนครปฐม

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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อสำรวจการติดเชื้อ *มัยโคพลาสมา ซินโนวีอี* ในไก่ผสมสามสายพันธุ์ที่เลี้ยงในเขตจังหวัดนครปฐมระหว่างเดือน กันยายน 2548 ถึง ตุลาคม 2549 จำนวน 30 ฝูง ระหว่างอายุ 1- 4.5 เดือน ไก่แต่ละตัวถูกนำมาเจาะเลือดเพื่อตรวจด้วยวิธีทางซีรัมวิทยา คือ ซีรัมเพลทแอกกลูติเนชัน (เอสพีเอ) และชุดทดสอบอีไลซา และถูกนำมาป้ายเชื้อเพื่อตรวจด้วยวิธีหาสารพันธุกรรม (พีซีอาร์) ของเชื้อเอ็มเอส ผลพบว่าได้ผลบวกด้วยวิธีทดสอบเอสพีเอ อีไลซา และพีซีอาร์ จำนวน 12 ฝูง 9 ฝูง และ 8 ฝูง ตามลำดับ โดยมี 4 ฝูงที่พบผลบวกทั้ง 3 วิธี เมื่อแบ่งกลุ่มการศึกษาออกตามช่วงอายุ ดังนี้ 1 เดือน 1-2 เดือน 2-3 เดือน และ 3-4.5 เดือน พบผลบวกด้วยวิธีการทดสอบเอสพีเอ อีไลซา และพีซีอาร์ ระหว่างร้อยละ 0-60, 0-60 และ 0-50 ตามลำดับ จากข้อมูลการศึกษาครั้งนี้ชี้ให้เห็นว่าไก่ผสมสามสายพันธุ์ที่มีอายุมากขึ้นมีโอกาสพบการติดเชื้อเอ็มเอสมากขึ้นด้วย โดยจะพบสารพันธุกรรมของเชื้อเอ็มเอสในฝูงไก่ที่มีอายุมากกว่า 2 เดือนขึ้นไป แม้จะไม่มีการแสดงอาการทางคลินิกออกมาให้สังเกตได้

คำสำคัญ : อีไลซา ไก่ผสมสามสายพันธุ์ *มัยโคพลาสมา ซินโนวีอี* พีซีอาร์ เอสพีเอ

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Introduction

Mycoplasma synoviae (MS) is known as a subclinical infection of the upper respiratory tract in poultry. The complication with Newcastle and/or infectious bronchitis infection causes airsacculitis (Kleven, 2003). Furthermore, infectious synovitis can be found in birds which have a systemic infection, leading to inflammation of the tendons or bursa sheath. Birds show clinical signs that include respiratory and/or lameness resulting in economic losses due to retarded growth, decrease in egg production, infertility and hatchability and ending with carcass condemnation (King et al., 1973; Kleven, 2003). Chickens, turkeys and guinea fowls are natural hosts. Pheasants, ducks, and geese are susceptible to experimental infection (reviewed by Kleven, 2003). All ages of chickens can be naturally infected, starting at 1 week old but the disease is commonly found at 4-16 weeks old (Kleven, 2003). The morbidity rate is high, possibly up to 100%, whereas the mortality rate is low ranging 1-10% as long as there is no secondary infection. Respiratory rales frequently occur in birds infected with MS alone. Live viral vaccines including Newcastle and/or infectious bronchitis possibly increase the severity of airsacculitis in MS infected birds, especially in broiler chickens. MS infected layer chickens apparently experience a drop in egg production up to 10% at the beginning or almost point of peak of laying for 6-10 weeks.

MS transmission occurs via horizontal that birds can be infected by MS contaminated materials (Marois et al., 2005) or by infected bird to normal bird and/or vertical transmission. For vertical transmission, MS organisms from hens can be transferred to their progeny (Kleven, 2003).

MS infection can be diagnosed by 2 major methods: serology and antigen detection. Serology can be assessed by serum plate agglutination (SPA) or rapid plate test (RPT) hemagglutination inhibition (HI) and enzyme linked immunosorbent assay (ELISA), whereas antigen detection uses culture and polymerase chain reaction

(PCR). For the culture method, a colony of mycoplasmas needs to be isolated and identified as a MS colony by an immunofluorescent antibody technique (Corstvet and Sadler, 1964; Talkington and Kleven, 1983). However, the culture method is time consuming and laborious. PCR detection is the simple, rapid and highly sensitive method for MS infection (reviewed by Kleven, 2003).

In Thailand, some broilers and broiler breeder farms have been found the MS infection; therefore, these infected farms have established a MS clean status of breeder flocks by prevention, control and a biosecurity program. As we know, most backyard chickens or mixed Thai native chickens are owned by small farm holders, who usually have inadequate biosecurity that easily introduces MS organisms into the farms. However, no reports of MS infection in mixed Thai native chickens have been determined. Therefore, this study was to determine the surveillance and monitoring MS status in mixed Thai native chickens. The data of this study will be useful for growers, veterinarians and servicemen to prevent and control MS infection on farms. The objective of this study was to investigate the surveillance of MS infection in mixed Thai native chickens in Nakornpathom province 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 1-4.5 months and having had general vaccination program including Newcastle disease and infectious bronchitis disease vaccines without MS vaccination during the September 2005-October 2006 period. Most flocks were in a healthy condition but only flock I.D. 4, 10, 12 and 24 showed mild respiratory signs while collecting samples. Individual birds were bled at the wing vein, swabbed at the choanal cleft and then the numbers of blood and swab samples were identified. The blood samples were separated for MS serology. The swab samples were inoculated into 2 ml of Frey's broth medium supplemented with 15% swine serum (FMS) (Kleven, 1998) and submitted to the laboratory for DNA detection by PCR technique.

MS serology:

SPA procedure: Fresh sera were tested with MS 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 could be observed. Negative and positive sera were also included in each test. Sera were then stored at -20°C for ELISA determination.

ELISA: Frozen sera were completely thawed at room temperature (25°C) before testing. All procedures were done at room temperature. Sera were tested with commercial test kits, ProFLOK® (Synbiotics Corporation, USA) following the manufacturers' instructions. Briefly, diluted sera were added into MS antigen-coated plate, incubated, washed then peroxidase-labeled anti-chicken antibody (conjugated antibody) was added. After incubation, the plate was washed then a substrate was added and, finally, the stop solution was added. The plate was read in an ELISA reader at 405-410 nm manufactured by Labsystems Multiskan MS Type 352, Finland. The optical density of the negative and positive controls and the samples was calculated then interpreted according to the manufacturers' instruction. For the interpretation of ELISA, titer levels 0-269, 270-743, and equal or higher than 744 were negative, suspicious and positive reactors, 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 15,000xg, washed with distilled water, followed by dilute pellete with distilled water, boiling for 10 min, then placed 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 MSL-1 (5'-GAAGCAAAATAGTGATATCA-3') and primer MSL-2 (5'-GTCGTCTCCGAAGTTAACAA-3') (Qiagen) 10 pmole each, Taq polymerase (Fermentas) 1.25 U, $MgCl_2$ 1.25 mM and DNA template 5 µl (250 ng). MG strain S6 (ATCC 15302) and MS strain WVU 1853 (ATCC 25204) were used as negative and positive controls, 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 MS-positive flocks tested by SPA, ELISA and PCR were 12 (flock numbers 4, 7, 8, 10, 12, 15, 17, 21, 22, 23, 24 and 27), 9 (flock numbers 2, 4, 10, 11, 12, 22, 24, 25 and 27) and 8 flocks (flock numbers 4, 8, 9, 10, 12, 17, 18 and 24), respectively. In addition, there were 15 suspected flocks tested by ELISA (flock number 1, 2, 9, 11, 13, 15, 17, 18, 21, 22, 23, 25, 26, 27 and 28). Overall, there were 4 flocks (flock numbers 4, 10, 12 and 24) that detected the positive reactor in all tests (Table 1). The percentage of positive results depending on age 1 month, 1-2 months, 2-3 months and 3-4.5 months tested by SPA, ELISA and PCR was 0-60%, 0-60% and 0-50%, respectively (Table 2).

Discussion

This study revealed that the mixed Thai native chickens raised by small farm holders in the area of Nakornpathom province were diagnosed as MS infection at older than 1 month. No tests could detect the positive reactors against MS infection at 1 month old. At 1-2 month old, the SPA and ELISA detected positive reactors against MS infection. Moreover, both tests showed the positive reactor in the same flock (flock I.D. 22). In this study, the SPA could more rapidly detect the positive reactor compared with the ELISA. 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). In contrast to Ewing et al. (1996), they found that the SPA may not be sensitive to detect the early stage of MS infection in some flocks, whereas the ELISA and PCR can detect them. Interestingly, Ewing et al. (1996) suggested that ELISA should be considered as a serologic screening in stead of SPA. Several reports suggested that the ELISA should be used as the screening test instead of the SPA (Higgins and Whithear, 1986; Opitz et al., 1983; Patten et al., 1984). The advantages of the SPA are more convenience in the field, rapid diagnosis, no requirement for special equipment and/or technicians. Unfortunately, the PCR procedure could not detect the MS DNA in birds aged 1-2 month contrasting with the SPA and ELISA. Regularly, the PCR procedure could detect MS antigen more rapid and sensitive in tissues and culture medium compared with isolation and identification (Salisch et al., 1998). The probable reason is due to collecting sample techniques or the very low numbers of MS organisms in the sample. The percentage of positive flocks of birds, aged over 2 months, detected by the SPA was the same as that detected by the ELISA, but only five of the thirteen flocks showed the same positive flocks (flock I.D. 4, 10, 12, 24 and 27). In addition, the flock I.D. 8 and 17 that MS infection was detected by the MS PCR and SPA, not ELISA indicating that the SPA was much more sensitive than the ELISA. Even though the SPA may not be suggested for use as the serologic screening test (Ewing et al., 1996), but the result of this study and the advantages of this test including simple, no machine requirement and rapid diagnosis shows that the SPA may be suitable to use in the field. At 1 month old, no antibody responses

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

Flock I.D.	Age (month)	Numbers of samples	Submission date	Number of positive flocks/total		
				SPA	ELISA (Suspected)	PCR
1	2	15	14/09/2005	0/15	0/14, (1/1)	0/15
2	3.5	15	21/09/2005	0/15	1/12, (3/3)	0/15
3	2	15	28/09/2005	0/15	0/15	0/15
4	4.5	15	28/09/2005	15/15	14/14, (1/1)	14/15
5	1	15	28/09/2005	0/15	0/15	0/15
6	2	15	09/11/2005	0/15	0/15	0/15
7	1.7	15	16/11/2005	15/15	0/15	0/15
8	3	15	07/12/2005	2/15	0/15	7/15
9	3	15	07/12/2005	0/15	0/14, (1/1)	15/15
10	3	15	07/12/2005	15/15	14/15	3/15
11	3	15	07/12/2005	0/15	1/14, (1/1)	0/15
12	3.5	15	23/07/2005	15/15	12/12, (3/3)	14/15
13	2	15	11/01/2006	0/15	0/12, (3/3)	0/15
14	2	15	18/01/2006	0/15	0/15	0/15
15	1.5	15	31/03/2006	3/15	0/14, (1/1)	0/15
16	2	15	04/04/2006	0/15	0/15	0/15
17	3	15	11/04/2006	9/15	0/14, (1/1)	1/15
18	2	15	20/04/2006	0/15	0/12, (3/3)	3/15
19	1	15	20/04/2006	0/15	0/15	0/15
20	1	15	26/04/2006	0/15	0/15	0/15
21	2	15	01/05/2006	1/15	0/14, (1/1)	0/15
22	1.5	15	16/05/2006	3/15	1/8, (7/7)	0/15
23	4	15	19/07/2006	2/15	0/9, (6/6)	0/15
24	4	15	16/082006	15/15	14/14, (1/1)	14/15
25	3	15	30/08/2006	0/15	2/10, (5/5)	0/15
26	1	15	06/09/2006	0/15	0/14, (1/1)	0/15
27	2.5	15	28/09/2006	6/15	3/8, (7/7)	0/15
28	3	15	19/10/2006	0/15	0/13, (2/2)	0/15
29	1	15	27/10/2006	0/15	0/15	0/15
30	1	15	27/10/2006	0/15	0/15	0/15

Table 2 Percentage of positive results depending on age: 1 month, 1-2 months, 2-3 months and 3-4.5 months tested by SPA, ELISA and PCR.

Age (month)	Number of flocks	Number of positive flocks (%)		
		SPA	ELISA	PCR
1	6	0	0	0
1-2	11	4 (36.4%)	1 (9.1%)	0
2-3	8	4 (50%)	4 (50%)	4 (50%)
3-4.5	5	3 (60%)	3 (60%)	2 (40%)

and/or evidence of MS infection were observed in this study. The incubation period of MS generally ranges 11-22 days from exposure contact study (Bradbury et al., 1994). Therefore, birds infected at least 22 days will be present the evidence of infection. Moreover, natural infection should take longer than the exposure contact study. These reasons explain why the evidence of infection and clinical signs are not observed at 1 month old.

Regarding sampling history, positive reactors were detected by SPA, ELISA and PCR in the older flocks; respiratory signs including coughing, sneaking, conjunctivitis and increased lacrimation were observed only in flock I.D.4, whereas flocks I.D. 10, 12 and 24 showed mild degrees of respiratory signs. Although some flocks showed mild degrees of respiratory signs, they presented more severe respiratory signs if they had received live Newcastle and/or infectious bronchitis virus vaccine. Interestingly, a higher chance for MS infection was observed in older flocks. The possible reason is that the management and biosecurity of the positive farm were not efficient. MS organisms can be transmitted by contaminated vectors including growers, equipment, etc. Christensen et al. (1994) found that MS organisms can sustain their viability on feathers and in the nasal passage for 3 days and 12 hours, respectively. Therefore, effective farm management and biosecurity can prevent MS infection and reduce the cost of the therapeutic treatment.

The PCR procedure could firstly detect the MS DNA at 2 months old. For example, the flock I.D. 18 found the MS DNA 3 out of 15 samples, but did not reveal the antibody reactor detected by either SPA test or ELISA or any clinical signs, indicating the early MS infection of this flock. In this study, the MS infection was not observed at 1 month old but was observed starting at 1.5 month old. The possibly reason is good farm management and biosecurity during the young birdís life or the brooding period compared with that of older bird rearing, leading to the low chance of MS infection in young birds.

In surveillance of *Mycoplasma synoviae* infection in mixed Thai native chickens in the area of Nakornpathom province. During the September 2005 to October 2006 period, 30 flocks, aged between 1 and 4.5 months determined by SPA test, ELISA and PCR procedure were investigated. The positive reactors detected by the SPA test, ELISA and PCR procedure were 11, 8 and 6 flocks, respectively. This study indicates that in flocks infected with MS organisms, birds may not show clinical respiratory signs. In addition, the older the flock the higher number of positive reactors found. The SPA test is appropriate to monitor the surveillance of MS infection in suspected flocks.

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