Eradication of *Mycoplasma synoviae* from a multi-age broiler breeder farm using antibiotics therapy

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ABSTRACT *Mycoplasma synoviae* (MS) is a common respiratory pathogen in the poultry industry. Eradication of MS from broiler breeder flocks is important for reducing economic losses caused by MS-associated diseases on broiler farms. An outbreak of MS infection was detected on a multi-age Korean broiler breeder farm that implements a flock replacement program every 3 to 6 months and uses A-type cages. Continuous administration of tilmicosin after 2 rounds of intensive antibiotics treatment with chlortetracycline, doxycycline, and enrofloxacin reduced MS shedding from the MS-positive flocks and eventually eradicated MS from the farm. Flock 3 was the last MS-positive flock by both PCR and enzyme-linked immunosorbent assay (ELISA), while flocks introduced to the farm after flock 3 were MS-negative by both PCR and ELISA at the time of depletion. This is the first report of successful eradication of MS from a multi-age broiler breeder farm using continuous antibiotic treatments.

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INTRODUCTION

Mycoplasma synoviae (MS) is a major pathogenic mycoplasma in chickens. MS infection of chickens frequently occurs as a subclinical upper respiratory disease, but it can cause severe airsacculitis or synovitis under conditions of co-infection with other viral or bacterial infections (Kleven et al., 1972; Macowan et al., 1982; Droual et al., 1992; Senties-Cue et al., 2005). MS can be transmitted vertically from parents to their offspring, and in a previous study, broiler flock obtained from MS-seropositive broiler breeders showed higher rates of mortality for airsacculitis (King et al., 1973; MacOwan et al., 1984). MS infected broiler flocks in the field showed reduced weight gain, poorer feed conversion, and increased rates of mortality for airsacculitis (King et al., 1973). Most broiler breeder farms consider implementing an eradication program against MS. In a previous study, 62/70 broiler breeder flocks in Korea were seropositive for MS (Cho et al., 2014). Although a temperature-sensitive attenuated MS vaccine, MS-H, has been developed and used worldwide (Morrow et al., 1998; Kleven, 2008), the vaccine is not yet available in Korea. MS is however sensitive to many antibiotics, including chlortetracycline, oxytetra-cycline, tylosin, tilmicosin, and tiamulin (Kleven and Anderson, 1971; Olson and Sahu, 1976; Jordan and Horrocks, 1996; Wang et al., 2001; Cerda et al., 2002; Landman et al., 2008). In a previous study, eradication of MS from a single-age broiler breeder farm was achieved using intensive antibiotic treatments with fluoroquinolones and oxytetracycline (Fiorentin et al., 2003). However, to our knowledge there has been no report of eradication of MS from a multi-age breeder farm. In this article, we report the eradication of MS from a multi-age broiler breeder farm with intensive antibiotics treatment with tilmicosin, chlortetracycline, doxycycline, amoxicillin, and enrofloxacin.

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MATERIALS AND METHODS

History and Condition of the Broiler Breeder Farm

The Ross grandparent stock (**GP**) distributor in Korea, Samhwa Breeding Agri., Inc., has broiler breeder farms and a GP farm in separate locations. The broiler breeder farms obtain chickens from the GP farm run by the same company. The GP flock was free of mycoplasmas. *Mycoplasma gallisepticum* (MG)/MS enzyme-linked immunosorbent assays (**ELISA**) were performed regularly on one-day-old and 7-, 10-, 14-, 18-, 22-, 26-, 30-, 36-, 42-, 48-, 54-, and 60-week-old birds of the GP flock. One of the broiler breeder farms showed chronic MS infection without a reduction of egg pro-duction. The MS-infected Korean broiler breeder flock showed a slightly higher mortality, with gross lesions of *Escherichia coli* peritonitis (Raviv et al., 2007). The

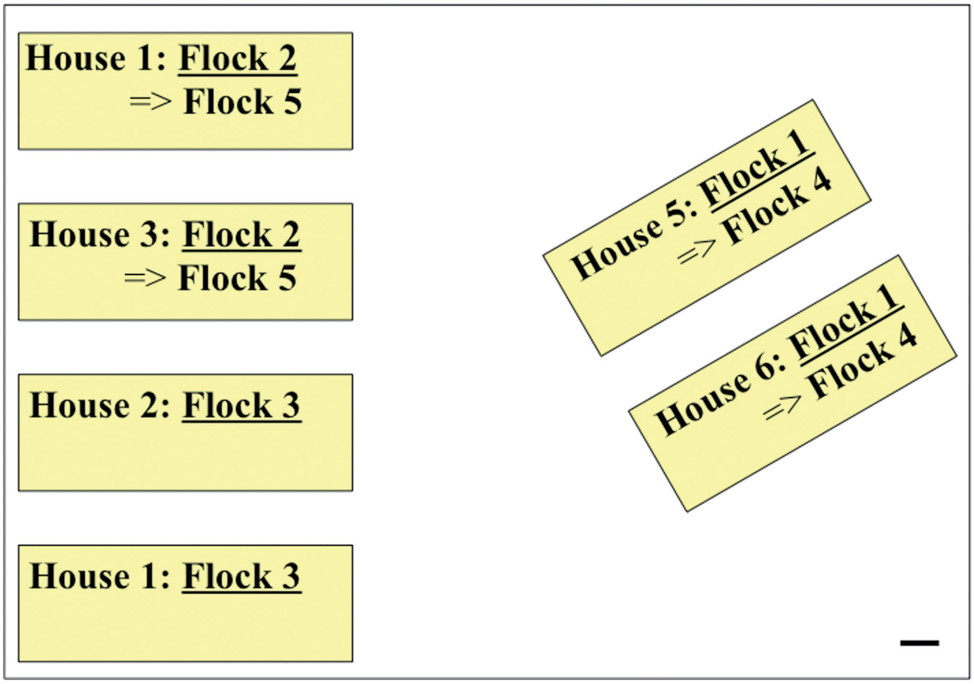

Figure 1. Locations of houses and a schematic of the flock replacement program of the broiler breeder farm. Scale bar = 20 m.

breeders on the farm were also infected with *Salmonella* Enteritidis. The farm has 6 houses, each of which can hold 10,000 birds. Two houses (20,000 of 10 to 14 week old brids) are replaced every 3 to 6 months (Figure 1). Four lines of A-type cages with hopper feeders and nipple drinkers are installed in each house. Scrapers are used for removal of feces every day. A vehicle disinfec-tion unit with a spray nozzle is installed on the farm, and the tires of the vehicle are soaked in disinfectant be-fore passing through the main gate of the farm. All visi-tors and workers must take a shower and change clothes before entering the farm. All equipment and vials must pass through a formaldehyde disinfection unit. Farm workers are separated, taking care only of their own house and not involved in work for the other houses, but they share an office and canteen. All workers enter the facilities after spray disinfecting the whole body, sanitizing their hands with 70% alcohol, and dipping their boots into disinfectant.

Antibiotics Treatments

Chlortetracycline at 10 mg/kg of body weight was introduced as a feed addition for 3 or 14 days. Treatments with 10 mg/kg of body weight of doxycycline or 10 mg/kg of body weight of enrofloxacin were performed via drinking water for 3 or 5 days. Amoxicillin at 6 mg/kg of body weight or tilmicosin at 7.5 mg/kg of body weight was administered via drinking water for 3 days. The history of treatments was summarized in Table 1. Gentamicin injection was also used during the week to control *Salmonella* infection. Total amounts and cost of antibiotics used in this study were summarized in Table 2.

MS-Specific PCR Assay

Every 2 weeks, 10 tracheal swabs and a feather sample were collected from each flock and delivered to the laboratory within 24 hours. The samples were inoculated into modified Frey broth with NAD and incubated for 24 to 36 hours at 37 °C. Twenty microliter aliquots of each culture were pooled and inoculated into 200 μL of modified PPLO broth with NAD and incubated for 1 week at 37°C. Bacterial genomic DNA was extracted from the pooled culture for each flock, and MS-specific PCR was performed according to a previously reported method (Ramirez et al., 2006).

MS-Specific ELISA

To detect MS-specific antibodies on the farm, 10 blood samples were collected every month from all flocks. MG/MS-specific ELISA (BioChek Ltd., Netherlands) was performed in accordance with the manufacturer's instructions.

RESULTS

The history of antibiotic treatments and the PCR and ELISA results are summarized in Table 1. Flocks 1 and 2 showed high levels of seropositivity against MS, and MS was sometimes detected in the flocks by PCR assay. In order to control MS infection in the flocks, chlortetracycline, doxycycline, and enrofloxacin were administered intensively at weeks 11 and 12; however, this treatment was not effective. After this 2 week administration, the ratio of positive results of MS-specific PCR started to decline, but most sera still contained

Table 1. Longitudinal changes over 71 weeks, according to time, age, treatment, and test result.

Weeks[a]	Antibiotics[b] (number of treatment days, mg/kg of body weight)	Flocks 1			2			3			4			5		
		Age[c]	PCR	ELISA	Age[c]	PCR	ELISA	Age[c]	PCR	ELISA	Age[c]	PCR	ELISA	Age[c]	PCR	ELISA
1	Til. (3, 7.5)	43	+		18	+										
6	Til. (3, 7.5)	48	–		22	–										
8	CTC (3, 10)	50		9/10	24		10/10									
11	CTC (14, 10)	53	–		27	+		13	–							
12	Enro. (5, 10) Doxy. (5, 10)	54	–	10/10	28	+	10/10	14	–	0/10						
15					31	+	9/10	17		0/10						
20			Depleted		36	–	10/10	22	–	0/10						
22	Doxy. (5, 10)															
24	Enro. (5, 7)				40	+	10/10	26	+	1/10						
28	Til. (3, 7.5)				44	+	10/10	30	+	3/10	15	+				
33	Til. (3, 7.5)				49	+	10/10	35	+	7/10	20	+	0/10			
37					53	–	10/10	39	–	7/10	24	–	0/10			
39	Til. (3, 7.5)				56			42			26					
40	Doxy. (3, 10)				57	–	10/10	43	–	9/10	27	–	0/10			
43	Enro. (3, 10)				60	+		46	–		30	–				
45	Til. (3, 7.5)				62	–	10/10	48	–	10/10	32	–	0/10			
47	Amo. (3, 6)				64			50			34					
51	Til. (3, 7.5)					Depleted		54		10/10	38		0/10			
52	Doxy. (3, 10)							55	–	10/10	39	–	0/10			
56	Enro. (3, 10)							59	–		43	–				
57	Til. (3, 7.5)							60			44					
58								61	–	8/10	45	–	0/10			
60									Depleted		47	–				
62											49	–	0/10	14	–	0/10
64											51	–		16	–	
68											53	–		18	–	
71											56	–	0/10	21	–	0/10

[a]Time in weeks since first flock was treated for MS on the farm.
[b]Til, Tilmicosin; CTC, Chlortetracycline; Doxy, Doxycycline; Enro, Enrofloxacin; Amo, Amoxicillin.
[c]Age is represented as weeks old.

Table 2. Amount and cost of antibiotics used in this study.

Antibiotics[a]	Duration (days)	Number of birds per treatment	Antibiotics amount	Total Cost (USD)	Cost/bird (USD)
Til	24	47,500	37 L	27,360	0.576
CTC	17	40,000	1,000 kg	4,250	0.106
Enro	16	55,000	528 L	10,560	0.192
Doxy	16	50,000	960 L	22,080	0.441
Amo	3	60,000	13.5 kg	243	0.004
Gent	1	40,000	500 ml	60	0.002
Total	77	292,500		64,553	1.321

[a]Til, Tilmicosin; CTC, Chlortetracycline; Doxy, Doxycycline; Enro, Enrofloxacin; Amo, Amoxicillin; Gent, Gentamicin.

MS-specific antibodies (Table 1). There was then a pause between antibiotic treatments for 10 weeks. After this pause, doxycycline, enrofloxacin, and tilmicosin were administered for 11 weeks (from weeks 22 to 33). However, the second round of intensive antibiotic treatments did not eradicate MS from flock 2, which was infected by MS after introduction to the farm. Instead, the newly introduced, MS-negative flock 3 turned into an MS-positive flock during the second round of antibiotics treatments, and the flock was seropositive for MS until depletion. In contrast to flock 3, flock 4, introduced to the farm after tilmicosin treatment, was MS PCR positive twice at an early age but became MS PCR negative at 24 weeks old until depletion. In addition, even though the flock was MS positive at an early age, MS specific antibodies were not detected at any point till depletion. Tilmicosin was administered continuously via drinking water every 6 weeks beginning at week 33. During continuous treatments with tilmicosin, all samples showed negative results for MS-specific PCR. Flock 3 was the last MS-positive flock by both PCR and ELISA. After the time point of depletion of flock 3, no positive result was confirmed for 10 weeks for flocks 4 and 5, by either MS-specific PCR or ELISA. In addition 2 following flocks after flock 5 had been kept MS negative as well (data not shown). Total cost of antibiotics therapy was 64,553 USD (1.321 USD/bird) in the farm during this study (Table 2).

DISCUSSION

MS has been reported to be transmitted vertically and laterally (MacOwan et al., 1984; Ewing et al., 1998), and MS infection is detected more frequently on multi-age layer farms than on single-age layer farms (Dufour-Gesbert et al., 2006). Therefore, eradication of MS from multi-age broiler breeder farms may be more difficult than the previously reported eradication of MS from a single-age broiler breeder farm (Fiorentin et al., 2003). In this study, we report successful eradication of MS from a multi-age broiler breeder farm in Korea. Since MS had never been monitored in the farm before this study, we could not obtain MS infection history of the farm. It is unclear how or when the farm was infected by MS, however we suspect a continuous MS infection in the farm as it is a multi-age farm and there had been no eradication program on MS before. During the whole study, the GP flock remained MS negative. The multi-age broiler breeder farm was a cage farm, which was performing artificial insemination. In addition, a farm manager went around all houses every day. Therefore certain mechanical transmission of MS between houses could have happened, although there was also a possibility of airborne transmission of MS in the farm.

Due to the development of the live attenuated vaccine MS-H, eradication of MS has been practicable in many countries. However, in Korea, the vaccine has not yet been registered. Antibiotics treatments and biosecurity are the only options for control of MS infection in Korea. Previous reports have suggested that the antibiotics susceptibilities of avian mycoplasmas may be different between species and strains (Jordan and Horrocks, 1996; Stanley et al., 2001; Wang et al., 2001). MS quickly acquired high-level resistance to erythromycin and tylosin during in vitro passages, while resistance to tiamulin or oxytetracycline was not detected after 10 in vitro passages (Gautier-Bouchardon et al., 2002). MS gradually developed resistance to enrofloxacin during in vitro passages, and it persistently infected farms treated with enrofloxacin in other previous studies (Stanley et al., 2001; Gautier-Bouchardon et al., 2002; Le Carrou et al., 2006). MS was susceptible to chlortetracycline, doxycycline, and tilmicosin in other studies (Olson and Sahu, 1976; Jordan and Horrocks, 1996; Landman et al., 2008). In previous studies, antibiotics administered for a short duration reduced the MS infection level, but it was not enough to eradicate MS from the infected flocks (Stanley et al., 2001; Catania et al., 2010). In this study, intensive antibiotic treatments with chlortetracycline, doxycycline, and enrofloxacin were not successful at eradicating MS from the infected flock or stopping lateral transmission of MS to newly introduced flocks. However, continuous administration of tilmicosin successfully eradicated MS from the farm, as in the previous study where continuous administration of oxytetracycline eradicated MS from a single-age broiler breeder farm (Fiorentin et al., 2003).

Flock 4 was MS PCR positive twice at an early age but MS specific antibodies were not detected at any point till depletion of the flock. There is a possibility that antibiotics treatment depresses antibody responses to MS infection in poultry.

We used huge amounts of antibiotics to control the MS infection in this study and the total cost of the antibiotic treatments was 64,553 USD. A decision to control MS using antibiotic therapy could be based on cost effectiveness. For example, if farmers, who own GP farms are willing to obtain an MS negative flock, they will be interested in this intensive antibiotic therapy. Otherwise, if farmers are unwilling to pay for such treatment to control MS infection, and neglect MS infection in their farms, then they will not be interested in antibiotic therapy. This successful eradication of MS might be achievable with fewer treatments if there is no concurrent *Salmonella* Enteritidis infection in the flock.

This is the first report of successful eradication of MS from a multi-age broiler breeder farm by use of antibiotic treatments. This study suggests that continuous treatment with antibiotics, to which MS is not able to rapidly develop resistance, might be useful for retarding the growth of MS and eventually eradicating MS from multi-age broiler breeder farms.

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REFERENCES

Catania, S., D. Bilato, F. Gobbo, A. Granato, C. Terregino, L. Iob, and R. A. Nicholas. 2010. Treatment of eggshell abnormalities and reduced egg production caused by Mycoplasma synoviae infection. Avian diseases 54:961–964.

Cerda, R. O., G. I. Giacoboni, J. A. Xavier, P. L. Sansalone, and M. F. Landoni. 2002. In vitro antibiotic susceptibility of field isolates of Mycoplasma synoviae in Argentina. Avian diseases 46: 215–218.

Cho, J. K., J. H. Kim, S. H. Kwon, W. Kim, C. K. Park, and K. S. Lim. 2014. Sero-epidemiological survey of egg-transmitted bacte-rial diseases in broiler breeder flocks in Korea. J Poult Sci 51:435–443.

Droual, R., H. L. Shivaprasad, C. U. Meteyer, D. P. Shapiro, and R. L. Walker. 1992. Severe mortality in broiler chickens associated with Mycoplasma synoviae and Pasteurella gallinarum. Avian diseases 36:803–807.

Dufour-Gesbert, F., A. Dheilly, C. Marois, and I. Kempf. 2006. Epidemiological study on Mycoplasma synoviae infection in layers. Veterinary microbiology 114:148–154.

Ewing, M. L., K. C. Cookson, R. A. Phillips, K. R. Turner, and S. H. Kleven. 1998. Experimental infection and transmissibility of Mycoplasma synoviae with delayed serologic response in chickens. Avian diseases 42:230–238.

Fiorentin, L., R. A. Soncini, J. L. da Costa, M. A. Mores, I. M. Trevisol, M. Toda, and N. D. Vieira. 2003. Apparent eradication of Mycoplasma synoviae in broiler breeders subjected to intensive antibiotic treatment directed to control Escherichia coli. Avian pathology : journal of the W.V.P.A 32:213–216.

Gautier-Bouchardon, A. V., A. K. Reinhardt, M. Kobisch, and I. Kempf. 2002. In vitro development of resistance to enrofloxacin, erythromycin, tylosin, tiamulin and oxytetracycline in Mycoplasma gallisepticum, Mycoplasma iowae and Mycoplasma synoviae. Veterinary microbiology 88:47–58.

Jordan, F. T., and B. K. Horrocks. 1996. The minimum inhibitory concentration of tilmicosin and tylosin for mycoplasma gallisepticum and Mycoplasma synoviae and a comparison of their efficacy in the control of Mycoplasma gallisepticum infection in broiler chicks. Avian diseases 40:326–334.

King, D. D., S. H. Kleven, D. M. Wenger, and D. P. Anderson. 1973. Field studies with Mycoplasma synoviae. Avian diseases 17:722–726.

Kleven, S. H. 2008. Control of avian mycoplasma infections in commercial poultry. Avian diseases 52:367–374.

Kleven, S. H., and D. P. Anderson. 1971. In vitro activity of various antibiotics against Mycoplasma synoviae. Avian diseases 15:551–557.

Kleven, S. H., D. D. King, and D. P. Anderson. 1972. Airsacculitis in broilers from Mycoplasma synoviae: effect on air-sac lesions of vaccinating with infectious bronchitis and Newcastle virus. Avian diseases 16:915–924.

Landman, W. J., D. J. Mevius, K. T. Veldman, and A. Feberwee. 2008. In vitro antibiotic susceptibility of Dutch Mycoplasma synoviae field isolates originating from joint lesions and the respiratory tract of commercial poultry. Avian pathology : journal of the W.V.P.A 37:415–420.

Le Carrou, J., A. K. Reinhardt, I. Kempf, and A. V. Gautier-Bouchardon. 2006. Persistence of Mycoplasma synoviae in hens after two enrofloxacin treatments and detection of mutations in the parC gene. Veterinary research 37:145–154.

MacOwan, K. J., M. J. Atkinson, M. A. Bell, T. F. Brand, and C. J. Randall. 1984. Egg transmission of a respiratory isolate of Mycoplasma synoviae and infection of the chicken embryo. Avian pathology : journal of the W.V.P.A 13:51–58.

Macowan, K. J., C. J. Randall, H. G. Jones, and T. F. Brand. 1982. Association of Mycoplasma synoviae with respiratory disease of broilers. Avian pathology : journal of the W.V.P.A 11:235–244.

Morrow, C. J., J. F. Markham, and K. G. Whithear. 1998. Production of temperature-sensitive clones of Mycoplasma synoviae for evaluation as live vaccines. Avian diseases 42:667–670.

Olson, N. O., and S. P. Sahu. 1976. Efficacy of chlortetracycline against Mycoplasma synoviae isolated in two periods. Avian diseases 20:221–229.

Ramirez, A. S., C. J. Naylor, P. P. Hammond, and J. M. Bradbury. 2006. Development and evaluation of a diagnostic PCR for Mycoplasma synoviae using primers located in the intergenic spacer region and the 23S rRNA gene. Veterinary microbiology 118:76–82.

Raviv, Z., N. Ferguson-Noel, V. Laibinis, R. Wooten, and S. H. Kleven. 2007. Role of Mycoplasma synoviae in commercial layer Escherichia coli peritonitis syndrome. Avian diseases 51:685–690.

Senties-Cue, G., H. L. Shivaprasad, and R. P. Chin. 2005. Systemic Mycoplasma synoviae infection in broiler chickens. Avian pathology : journal of the W.V.P.A 34:137–142.

Stanley, W. A., C. L. Hofacre, G. Speksnijder, S. H. Kleven, and S. E. Aggrey. 2001. Monitoring Mycoplasma gallisepticum and Mycoplasma synoviae infection in breeder chickens after treatment with enrofloxacin. Avian diseases 45:534–539.

Wang, C., M. Ewing, and S. Y. Aarabi. 2001. In vitro susceptibility of avian mycoplasmas to enrofloxacin, sarafloxacin, tylosin, and oxytetracycline. Avian diseases 45:456–460.