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Control of *Mycoplasma gallisepticum* Infection in Commercial Broiler Breeder Chicken Flocks Using Tilmicosin (Provitil Powder®) Oral Formulation

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Abstract: Despite the great efforts poultry breeding companies made towards eradication of pathogenic mycoplasmas from poultry flocks, Still *Mycoplasma gallisepticum* (MG) infection is of continuing economic concern in commercial broiler breeder chicken flocks. Control of MG infection in broiler breeder chicken flocks by chemotherapy is the most practical way to minimize economical losses. Tilmicosin is one of the most important antimicrobial agent used for treatment and control of MG infection. This study was carried out on eight broiler breeder chicken flocks with a history of MG challenge {signs, post mortem, and serology [enzyme linked immunosorbent assay (ELISA)]}. Tilmicosin (Provitil powder® 30 mg/kg body weight) was used to limit MG infection in these flocks, and was given to birds for 3 successive days every 5 weeks for 4 months. 136 serum samples were collected from these flocks (16-18 samples per flock) before starting tilmicosin treatment and the same number of serum samples were collected following each three treatment course at 5 weeks interval. *Mycoplasma gallisepticum* ELISA was performed on these samples for the purpose of detecting MG antibodies in these flocks and analyzing data subsequently. The first and second course of tilmicosin treatment did not have any significant effect on MG ELISA results (GMT) ($P>0.05$), while after the third course, GMT had dropped significantly ($P<0.05$) in all tested flocks compared to results gained prior starting treatment with tilmicosin. The overall sero-prevalence of MG in broiler breeder chicken population involved in the study was 80.43% and 44.09% before and after completing medication with tilmicosin respectively, while the percentage of antibody positive chickens among flock samples ranged from 38.88-100% and 18.75-56.25% before and after complete medication with tilmicosin respectively ($P<0.05$). Improvement in reproductive performances was observed (decreased mortality, increased feed intake and increased egg production and hatchability). The overall data presented here indicated that Provitil® (30 mg/kg body weight for 3 successive days every 5 weeks for 4 months) is considered a highly effective therapeutic in MG control programs for broiler breeder chicken flocks, and has a net positive effect for the producer. Further studies are necessary to assesses economic losses due to MG and the cost benefit of countermeasures.

Key words: *Mycoplasma gallisepticum*, broiler breeder, tilmicosin, enzyme linked immunosorbent assay

Introduction

Mycoplasma gallisepticum (MG) infection is responsible for significant economical losses in commercial broiler breeder chicken flocks industry (Carpenter *et al.,* 1979, Yoder, 1991; Evans and Hafez, 1992). Typically, infected flocks with MG exhibit increased mortality, increased feed conversion ratios, decreased egg production and hatchability, and respiratory disease (Yoder, 1991; Kleven, 1998; Levisohn and Kleven, 2000). Clinical signs may be exacerbated by viruses or bacteria such as a paramyxovirus or *Haemophilus paragallinarium* (Yoder, 1991). Production losses between 10 and 20% have been reported in layers and broiler breeder chicken flocks infected with MG (Bradbury, 2001). *Mycoplasma gallisepticum* infections are transmitted both horizontally and vertically and it remains in the flock constantly as sub clinical form (Bencina *et al.*, 1988a). Attempts to control this organism must be balanced against the need for greater efficiency in production costs. Control of avian mycoplasmosis infection by vaccination is limited as only few vaccines are available. Total eradication through test and slaughter is the most effective control method (Yoder, 1991), not only this is expensive but also the emergence of multiage complexes in the commercial layer industry makes this approach impractical (Evans and hafez, 1992; Levisohn and Kleven, 2000). Control of MG infection in broiler breeder by chemotherapy is the most practical way to minimize economical losses. The most important antimicrobial agent used for treatment and control of MG infection is tilmicosin. Tilmicosin is a broad-spectrum bacteriostatic synthesized from tylosin for veterinary use only. It has an antibacterial spectrum that is predominantly effective against *Mycoplasma* spp, *Pasteurella* spp, and various

Gram-positive organisms (Prescott, 2000). It has been used extensively to treat respiratory disease in swine, cattle and sheep (Moore *et al.*, 1996; Hoar *et al.*, 1998; Christodoulopoulos *et al.*, 2002) Tilmicosin is licensed for treatment and control of respiratory diseases associated with MG, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale* and *Pasteurela multacida* in chickens (Jordan and Horrocks, 1996; Kempf *et al.*, 1997; EMEA, 1998; Varga *et al.*, 2001). The present study was conducted to evaluate the effects of giving tilmicosin (Provitil® powder) at a dose rate of 30 mg/kg body weight (bw) for 3 successive days repeated every 5 weeks for 4 months to broiler breeder chicken flocks exposed to MG.

Materials and Methods

Flocks and study area: This study was conducted during the period from November 2005 - February 2006 in Jordan. A total population of 36,000 broiler-breeder chickens was distributed over 8 operating broiler-breeder farms. The age of flocks was 19-43 weeks before treatment with tilmicosin had started. Each farm had 1–2 houses and these houses were built from brick and cement with metal-plate roofs and were of different sizes. Farms followed an open-house system. The stocking density was 5 birds/m^2. Birds were housed in an intensive deep-litter system. Before birds were placed, houses were cleaned, washed, disinfected and provided with new wood shavings. Four of breeder flocks were imported from France, 2 from Germany and the rest flocks from Netherlands. Feeding programs were designed according to the breeding company (grand parent) manual instructions or management guide. Flocks were kept for a production life of 72–84 weeks, and then sold for slaughter. The breeds were Ross (4 flocks), Lohman (2 flocks) and Hubbard (2 flocks). Broiler Breeder growers in Jordan adopt health programs that include bacterial and viral disease vaccines.

Flocks history: None of the breeder flocks included in this study had received any vaccination against MG. The majority of these flocks had exhibited a decrease in feed intake, egg production (10-11%) and hatchability (8%) and increase in mortality (3%) over a period of less than 1 wk. On physical examination, birds appeared healthy and had good flesh, with a slight suborbital sinus swelling noted. Tracheitis, mild airsacculitis and salpingitis were seen on necropsy. All flocks included in this study were tested by ELISA for the presence of MG antibodies at one day old and were found negative.

Blood sample: All eight broiler breeder chicken flocks chosen for performing this study were contacted through appropriate veterinarians. Each farm in the study was visited four times; the first was just before starting tilmicosin treatment and the other three visits were carried out every 5 weeks of treatment. 136 serum samples were collected at each visit (16-18 samples per each flock) and questionnaires were filled. Each house in the farm was given a certain number then a number was drawn from each farm to be sampled. Three parallel imaginary lines were drafted along the length of the house (left, right and center) 5-6 birds were picked up along one walk covering the length of the house using each of the three lines, then blood was collected from these birds by veno-puncture of the wing vein. All blood samples were collected from these flocks were submitted immediately to Provimi Jordan Laboratories (ISO 17025 accredited) for analysis.

Drug: Provitil® (tilmicosin phosphate powder (300 mg/gm), Provimi Jordan, Amman Jordan) was used in this study. Provitil® was given according to manufacturer instructions (30 mg/kg body weight for 3 successive days). The recommended dose was repeated every 5 weeks for 4 months.

Enzyme-linked immunosorbent assay (ELISA): The serum samples were given a specific code number for each flock, and were kept frozen at -20°C till until analysis. A commercial test kit was used to detect specific antibodies against MG based on indirect enzyme linked immunosorbent assay (Synbiotics Corporation, ProFlock KPL, USA). A final serum dilution of 1:100 was used according to the manufacturer instructions. All collected serum samples from each flock (before and three course of medication with tilmicosin) were run in the same test plate to prevent conclusions error that may due to day variation of the test (Kreider *et al.*, 1991 a,b). Positive and negative reference controls provided by the manufacturer were also used in each test run for quality control and confirmation of the results. Optical density values were set at 405 nm wave length using an ELx800 ELISA reader (BIO-TEK Instruments, Inc. Winooski, VT, USA).

Statistical analysis: ELISA geometric mean titer (GMT) before, first, second, and third treatment with tilmicosin were compared by means of the Chi-square test. A significant level of 5% was used. Statistical analysis was performed with the SAS 8.2 package (SAS Institute, 1999).

Results and Discussion

None of the flocks involved in the current study were less than 5 weeks of age nor were they vaccinated against MG. Therefore, antibodies detected in the first set of sera indicate a field exposure to MG and exclude that the detected antibodies are maternal antibodies or resulted from vaccination.

The majority of flocks (No. 2, 3, 5, 6, 7, 8), had high

Table 1: *Mycoplasma gallisepticum* ELISA geometric mean titer (GMT) and sero-prevelence before and after first, second and third treatment trials using Provitil® (tilmicosin powder) at a dose rate of 30 mg/kg bw for 3 successive days repeated every 5 weeks

Flock no.		Before treatment	First treatment trial	Second treatment trial	Third treatment trial
1	Age (weeks)	39	44	49	54
	no. of samples tested	16	16	16	16
	no. of positive samples	7	9	5	3*
	ELISA GMT	155	210	410	24*
	Sero-prevalence (%)	43.75	56.25	31.25	18.75*
2	Age (weeks)	43	48	53	58
	no. of samples tested	18	18	18	18
	no. of positive samples	17	17	14	7*
	ELISA GMT	1940	1722	1415	635*
	Sero-prevalence (%)	94.44	94.44	77.77	38.88*
3	Age (weeks)	19	24	29	34
	no. of samples tested	16	16	16	16
	no. of positive samples	14	15	11	8*
	ELISA GMT	2311	2410	1970	1206*
	Sero-prevalence (%)	87.5	93.75	68.75	50.00*
4	Age (weeks)	42	47	52	57
	No. of samples tested	18	18	18	18
	No. of positive samples	7	6	5	4*
	ELISA GMT	108	90	65	28*
	Sero-prevalence (%)	38.88	33.33	27.77	22.22*
5	Age (weeks)	22	27	32	37
	no. of samples tested	16	16	16	16
	no. of positive samples	15	16	12	8*
	ELISA GMT	1510	1345	1145	950*
	Sero-prevalence (%)	93.75	100	75	50*
6	Age (weeks)	40	45	50	55
	no. of samples tested	18	18	18	18
	no. of positive samples	16	14	12	9*
	ELISA GMT	1237	1121	1055	885*
	Sero-prevalence (%)	88.88	77.77	66.66	50*
7	Age (weeks)	39	44	49	54
	no. of samples tested	16	16	16	16
	no. of positive samples	16	16	13	9*
	ELISA GMT	3282	2212	1810	1288*
	Sero-prevalence (%)	100.00	100.00	81.25	56.25*
8	Age (weeks)	34	39	44	49
	no. of samples tested	18	18	18	16
	no. of positive samples	18	15	14	11*
	ELISA GMT	2227	1989	1757	1350*
	Sero-prevalence (%)	100.00	83.33	77.77	68.75*

**significantly different from before treatment ($P<0.05$).

geometric mean titers (GMT) (>1000) before starting tilmicosin treatment program. This indicated an infection with highly pathogenic strains of MG, while for flocks No.1 and 4, the GMT titers was low (<500) before starting treatment, which reflected either an initial stage of infection, or an exposure to low pathogenic MG strains. The first and second course of tilmicosin treatment did not have any significant effect on MG ELISA results (GMT) ($P>0.05$), while after the third course, GMT had dropped significantly ($P<0.05$) in all tested flocks compared to results gained prior starting treatment with tilmicosin (Table 1 and Fig. 1). The highest seroprevalence (100%) was in flocks no 7 and 8 before starting tilimicosin treatment, while after third treatment with tilmicosin, it dropped significantly into 56.25% for flock no.7 and 68.75% for flock no.8 ($P<0.05$). All tested

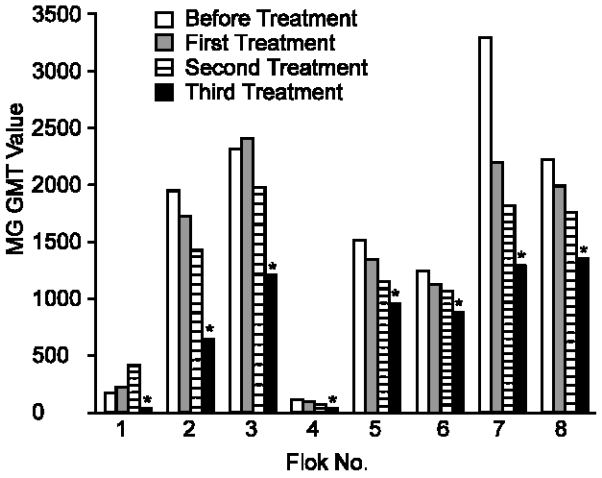

Fig 1: *Mycoplasma gallisepticum* ELISA geometric mean titer (GMT) before and after first, second and third treatment trials using Provitil® (tilmicosin powder) at a dose rate of 30 mg/kg bw for 3 successive days repeated every 5 weeks. *Significantly different from before treatment (P<0.05).

flocks exhibited a marked decrease in MG seropositive rates after completing the treatment program. The overall seroprevalence had dropped significantly from 80.43% to 44.09% before and after completing treatment (Tilmicosin 30 mg/kg body weight for 3 successive days every 5 weeks for 4 months) respectively (P<0.05). The rate of antibody-positive chickens among flocks ranged from 43.75%-100% and 18.75-66.67% before and after completing treatment with tilmicosin respectively (P<0.05) (Table 1).

During the period in which this study was conducted, there was a gradual increase in egg production (5-6 eggs per hen) in all flocks included in this study. This comes in agreement with previous reports on the effect of tilmicosin treatment on egg production in layers and breeder chicken flocks which resulted in producing 6 or more eggs per hen compared to non treated hen (Charleston *et al.*, 1984, Bradbury, 2001). Other criteria of reproductive performance such as hatchability, mortality, feed intake, and livability of progeny had also improved (data is not show).

The ELISA results in the current study provide evidence of the wide distribution of MG and of a considerably high incidence of infection among broiler breeder chicken flocks in Jordan; this comes in agreement with a previous study Roussan and Gharibeh (2005) which showed that the prevalence of MG in broiler, layer, and broiler breeder chicken flocks in Jordan were 70.4%, 80%, and 71.4% respectively. This high prevalence confirms the endemic nature of the disease in Jordan. *Mycoplasma gallisepticum* seroprevalence in chickens has been reported to be high in many countries with no control strategies or in countries before the implementation of control strategies. For example, MG seroprevalence was 73% in layers in Southern California in 1986 (Mohammed *et al.*, 1986). In Aragua State, Venezuela, seroprevalence of MG in layers was 59% and 66% in two different municipalities (Godoy *et al.*, 2001). There were reports of MG seroprevalence of 53% in broilers in Mongolia (Zhang et al., 2001), 47.5% in Zaria, Nigeria (Abdu *et al.*, 1993) and 10-13% in Croatia (Zelenika *et al.*, 1999).

A review of current literature reveals a number of articles suggesting the use of antibiotics to control poultry mycoplasmosis. Tilmicosin was among these antibiotics, were used effectively in treatment programs to reduce losses associated with mycoplasmosis (Evans and Hafez, 1992; Jordan and Horroks, 1996; Hoar *et al.*, 1998; Levisohn and Kleven, 2000). Abu Basha *et al.* (2006) investigated the pharmacokinetics and tissue concentrations of two formulations of tilmicosin (Provitil® [tilmicosin phosphate powder 300 mg/gm, Provimi Jordan, Amman Jordan] and Pulmotil® AC ([tilmicosin phosphate liquid 250 mg/ml, ELANCO Animal health, Div. Eli Lily, Geneva, Switzerland]), after oral administration of a single dose (30 mg/kg body weight) in chickens, they concluded that, tilmicosin was rapidly absorbed, slowly eliminated, and he also showed that the peak plasma concentration detected in chickens after oral administration of Provitil® (30 mg/kg body weight) was 2.12 ± 0.40 µg/ml. These values were higher than the minimum inhibition concentrations (MICs) for *Ornithobacterium rhinotracheal* (0.06-1 µg/ml) (Varga *et al.*, 2001) and *Mycoplasma gallisepticum* and *M. synoviae* (0.0125-0.1 µg/ml) and lower than MICs for *Clostridium perfringens* strains isolated from commercial broiler farms (Watkins *et al.*, 1997). Keles *et al.* (2001) investigated the pharmacokinetics and tissue concentrations after oral administration of a single dose of tilmicosin (50 mg/kg b.w.) in fowls and concluded that; tilmicosin was slowly eliminated from serum and lung with mean half-lives of 30.18 ± 2.38 and 75.74 ± 3.67 hour, respectively. The mean peak concentration of tilmicosin was 6.2 greater in the lung (7.96 ± 0.30 µg/ml) than that in serum (1.28 ±0.04 µg/ml) and reached peak concentration at 4.66 ± 2 and 17.78 ± 7.51hour, respectively. The NCCLS guidelines for tilmicosin susceptibility list a breakpoint of ≤ 8 µg/ml (Watts, 1999). This clearly demonstrates that the serum concentrations of tilmicosin are lower than the MICs for some susceptible bacteria. Nevertheless, several studies demonstrated that administration of tilmicosin at the recommended dose is effective for control of respiratory disease in several animal species (Moore *et al.*, 1996; Christodoulopoulos *et al.*, 2002). The high success rate of treatment is due to the prolong presence of therapeutic concentrations of tilmicosin in the lung

tissues (Papich and Riviere, 2001). The concentration of tilmicosin in rats' lungs was higher than the serum tilmicosin at all tested times and the infected rats with *Mycoplasma pulmonis* had higher lung tilmicosin concentration than non-infected rats (Modric *et al*., 1998). This phenomenon was also seen in lung tissues of chickens, swine and cattle (Scorneaux and Shryock, 1998a; 1998b; 1999).

The results of this study provided serological evidence on the effectiveness of using Provitil® (30 mg/kg body weight for 3 successive days repeated every 5 weeks for 4 months) in treatment and control of MG infection in broiler breeder chicken flocks and suggest a Mycoplasmacidal effect of tilmicosin which agrees with previous report on the high success rate of tilmicosin treatment due to prolonged presence of therapeutic concentration of tilmicosin in lung tissues (Papich and Riviere, 2001). The overall data presented here indicates that Provitil® (30 mg/kg body weight for 3 successive days every 5 weeks for 4 months) is considered a highly effective therapeutic in MG control programs for breeder chicken flocks, and has a net positive effect for the producer through slowed the rate of spread of MG infection within flocks, increasing in eggs production, and hatchability, and reducing mortality. However it is also important to consider eliminating other factors (stress, and viral problem) that may have a direct effect on flock health status.

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