

## Effect of Tilmicosin in Control of Mycoplasmosis in Broiler Chickens from Infected Breeders Using Elisa Test for Evaluation

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**Abstract:** Studying the effect of Tilmicosin (Pulmotil Ac) in the treatment and prevention of chorionic respiratory disease (CRD) in broiler was the purpose of this study. In this study, 10,000, 1 day old commercial broiler chicks from both Cobb and Ross reared in 4 houses; 2 Cobb and 2 Ross (5000 chicks each). The breeder and their chicks were positive to MG and MS. Tilmicosin was used as 75 mg/ liter at 35 weeks of age for 2 days with repletion at 20 day of age. Cobb breeder sera tested with serum plate agglutination (SPA) test were positive Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) (20 % and 26.7%), while Ross breeder was negative for MG and 23.33% positive results for MS. Furthermore, the 1 day old chicks tested sera were positive to MG in 10%, 15% 27.5 % and 27.5 and for MS in 30%, 25% , 37% and 30% ; respectively. Tilmicosin completely eliminate the serum positive titers for MG and partially eliminate it for MS as measured by Enzyme linked immunoassay (ELISA) titres and SPA test. In treated Cobb chicks MS ELISA titres still positive at the 17 days of age, while it was negative for MG. The recorded results of SPA test in treated flocks were generally lower than non treated ones. At PM examination the prevalence of marked air sac gross lesions in non treated control group indicated the development of CRD, the lesions increased in severity with age in non treated. The treated groups showed milder lesions varied from normal to slight turbidity without marked difference between medicated flocks. Generally; the livability taken in inconsideration the growth and mortality rates of treated flock was better than those of same non treated frocks. It can be concluded that usage of Tilmicosin can be essential in the treatment and prevention of CRD in broilers derived from positive breeders. In the other hand, the usage of such drug must be controlled to keep sensitivity of the organism

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**Key words:** Mycoplasmosis, control, Tilmicosin, broiler chickens, ELISA-test, SPA-test

### 1. Introduction

Both *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are the main cause of respiratory disease in chickens in combination with respiratory bacteria and viruses as well as bad environmental factors chronic respiratory disease (CRD) well be developed and characterized by respiratory signs, decreased growth and downgrading of carcasses at slaughter because of airsacculitis and increasing of total bacterial count (Neuman, et al. 1986 and Kleven, 1998). Therefore; CRD is one of the most important veterinary diseases all over the world. . Increased medication costs are additional factors for economic losses (Yoder, et al. 1977 and Ley and Avakian, 1992).

Antimycoplasma drugs including Tilmicosin (Pulmotil Ac) are widely used intensively to improve productivity of infected broiler flocks (Guarini, et al, 1999; Levisohn and Kleven ,2000; Saif et al, 2003, Amer et al., 2009a , b and Zakeri, and Kashefi, 2011). Also, Tilmicosin given at a dosage of 75 mg/litre administered to day-old chickens for 3 days,

under commercial conditions then a further treatment for 1 day was repeated after 4 weeks the drug was effective in controlling of mycoplasmosis in broiler (Guarini, et al ,1999; Scolari and Guarini. 1999 and Amer, et al 2009 a and b) and in the treatment of broiler breeders infected with Mg and Ms and had reduced their serological outcome as measured by ELISA titres (Saggiorato, et al, 2000; Talebi and Ghasemi-lak, 2004 and Wang-ZhiQiang et al ,2004).

Therefore, our field study was conducted evaluate the effect of Telmucosin in control of Mycoplasma in broiler flocks derived from commercial infected Ross and Cobb breeders reared. Antibodies to MG and MS were detected in treated chicken sera as compared with their control non treated flocks by ELISA test and SPA test to evaluate the efficacy of the used drug.

### 2. Material and Methods

#### Chicken flocks:

Ten thousands, 1 day old commercial broiler chicks from both Cobb and Ross were obtained from

breeder breeds having positive SPA results for both MG and MS.

**Ration:**

The chicks were feed on prepared ration according to the Ross broiler management manual and NRC (1984) requirement for broiler. All housed chickens were given ration adlibitum.

**Tilmicosin (Pulmotil AC®):**

Tilmicosin affects MG, MS, *Pasteurella multocida* and *Orinobacterium rhinotracheale*. It was used in a dose of 20 mg/kg b.w. in a concentration of 75 mg/Liter of drinking water for the 1<sup>st</sup> 2 days of life and repetition at the 19 days of age for anther 2 days as indicated by the producer and previous study (Amer, et al, 2009a).

**Detection of MG and MS in breeders:**

From each breeder flock 30 blood samples for serum were collected from hens and cockerels. The collected sera were diluted and subjected to SPA-test against colored MG and MS antigen according to Ewing et al, (1996) and Kleven (1998).

**Diagnosis of MG:**

MG infection was confirmed using Immuno-histochemistry using peroxidase detection kit purchased from Novocastra Co. lot No. 711010 on sections of tracheae and lungs from chicks with suspected signs and lesions as well as in sacrificed 40 chicks/ breed at 1 day and 35 days of age (Radi, et al, 2000).

**Serum sampled:**

From each broiler test flock 40 random blood samples were collected for serum at 1, 8, 17, 26 and 35 day of life. Samples collected at 1 day from sacrificed chicks while those collected at other times were taken from wing vein. Only 30 of highest quality serum samples/ flock/ time were subjected to ELISA testing against *Mycoplasma*.

**Serum Plate agglutination (SPA) test:**

Serum Plate agglutination (SPA) test SPA-test was carried out as 0.02 ml of serum was mixed with 0.02 ml of stained antigen of MG or MS, clumping indicates positive result (Ewing et al, 1996 and Kleven 1998). Stained M.G and MS antigens for SPA test were obtained from "Intervet International BV Boxmeer, Holland".

**Elisa test kits:**

Commercial mycoplasma MG and Ms antibody test kits was obtained from Synbiotics Corporation, San Diego, CA . 92127,USA. ELISA test procedures were done following the recommendations of the manufacturer with the assistance of full automatic plate washer Model ELX800 and ELISA Reader (Bio-Tek, ELX-800-650) were used.

**Air Sac Lesions:**

The air sacs of in dead and sacrificed chickens were examined according to (Guarini, et al 1999).

**Experimental design:**

At the 1<sup>st</sup> day of life, 40 chicks/ breed were scarified for serum with collection of tracheae and lungs from chicks with suspected lesions for Immuno-histochemistry. The rest of used chicks were equally divided into 2 groups (4990 chicks each). The chicks were reared in closed houses under similar management and environmental conditions.

Cubb chicks were kept as flock 1 and 2 while Ross chicks were kept as flock 3 and 4. Flocks 1 and 3 were left without treatment as control while those of 2 and 4 were medicated with Tilmicosin. All flocks were subjected to daily observation for general health condition. At the end of breeding period (35 days) 20 chicken/flock were scarified and examined for air sac lesions. The obtained results are shown in table (1 and 2).

**3. Results and Discussion**

The used chicks in this study were proved to be derived from MG and MS infected breeders by SPA resulting of sera. SPA tested Cobb breeder sera showed 20 % (6/30) positive MG and 26.7% positive MS (8/30), while Ross breeder was negative for MG and (7/30) 23.33% positive results for MS. Furthermore, the tested diluted sera of 1 day old chicks (Table 2) showed positive results to SPA test against stained antigen of MG in Cobb 4/40 (10%) and 6/40 (15%) as well as MS were 12/40 (30%) and 10/40 (25%). Additionally; MG positive sera in Ross were 11/40 (27.5%) and 11/40 (27.5%) but MS were 15/40 (37.5%) and 12/40 (30%); respectively. This result indicated that both mycoplasmas are prevalent in the breeder flocks reared in Egypt as smaller results were reported by Saif-Edin (1997).

Immuno- histochemistry testing of tracheal and air sac sections of chicks showing respiratory signs in form of gasping as well as air sac lesions were positive for MG (Kempf, et al. 1997a; Saif et al., 2003 and Amer e al, 2009a). The aforementioned results confirmed that the used chicks were vertically carrying mycoplasma infection as both mycoplasmas are vertically transmitted from dam hen to their progeny as diagnosis of Mycoplasmosis in breeder was passed on detection of antibodies by SPA or ELISA test and in 1 day old broilers chicks was confirmed by results of SPA and Immuno-histochemistry (Saif, et al 2003 and Amer e al, 2009a). There was a report by Saif-Edin, (1997) who found that the ELISA Examined serum samples from chicken flocks in Egypt, showed prevalence to against MG and MS as 100% in commercial broiler flocks and 40% in broiler parent flocks. Trawinska, et al. (2003) in Serological monitoring Cobb line reproductive hens with ELISA tests for the presence

of antibodies of MG and MS appeared at a small percentage (5%, 4.3%).

Both prevention and control programs, which may include surveillance (serology, culture, isolation and identification), vaccination and medication by using of antimycoplasma drugs account for additional costs (Carpenter et al. 1981 and Mohamed et al. 1987). Therefore; Tilmicosin was used for treatment and limitation of such infection in chicks under test (Levisohn and Kleven, 2000 and Talebi and Ghasemi-lak, 2004).

Tilmicosin completely eliminate the serum positive titers for MG and partially eliminate it for MS as measured by ELISA titres (table 1) and SPA test (table 2); when used at ratio 75 mg/ liter at 1<sup>st</sup> 3 days and 19 days of age for 2 days of age. Our results can be agree with those of Talebi and Ghasemi-lak (2004) who concluded that antibiotics affect the outcome of the Mg and Ms infections in broiler breeders and reduce serological titres of MG and MS infected birds but do not completely cure the birds from the infections, Levisohn and Kleven (2000) who reported that complete elimination of MG from all birds in an infected flock by mass antimicrobial therapy is an unrealistic expectation, and treatment should be regarded as a method for short-term amelioration of disease and economic effects.

In treated Cobb chicks MS ELISA titres still positive at the 17 days of age, while it was negative for MG (table 1). This observation could be explained by finding of Stipkovits (2000) who stated that MS strains have higher antibiotic resistance than

other avian mycoplasmas; therefore it is more difficult to treat infected flocks successfully. As well as Wang-ZhiQiang et al (2004) and Mavromati, et al. (2011) who stated that Tilmicosin was proven to be effective in the treatment of MG and MS infections in chicken when administered in drinking water.

The recorded results of SPA test (table 2) in treated flocks were generally lower than non treated ones. This result indicates the relation between both used tests as reported by Ewing (1996) who found that the MS seropositive flocks sera with SPA were 98.6 % positive for ELISA. The continuous detection of positive SPA during the study indicates that mycoplasma infection still circulating in the treated birds but in lower rates. This observation can be explored by the finding of Talebi and Ghasemi-lak (2004) as the tiamulin do not completely cure the birds from the infections.

At PM examination the prevalence of marked air sac gross lesions in non treated control group indicated the development of CCRD, the lesions increased in severity with age in non treated. The treated groups showed milder lesions varied from normal to slight turbidity without marked difference between medicated groups as recorded by Jordan and Horrocks (1996) and Stipkovits (2000) where the prevalence of gross lesions of the air sac was similar in all the medicated groups and was less than those of the infected non medicated. This result can be clarified by Talebi and Ghasemi-lak (2004) who reported the antibiotic do not completely cure the birds.

**Table 1.** Results of ELISA test for MG and MS in sera of treated and non treated Cobb and Ross broiler chickens.

Flock	Breed	Treatment	Days / days	MG	MS
				GMT± SD	GMT± SD
1	Cobb	Non	1	8491.9 2407.3	2961.0 2668.3
			8	1122.8 1761.2	832.3 1924.6
			17	819.30 567.43	25.0 843.6
			26	2.0 61.0	399.2 843.6
			35	0.0 0.0	1399.2 3264.9
2	Cobb	treated	1	8491.9 2407.3	2961.0 2668.3
			8	8.0 106.0	125.0 1264.9
			17	0.0 0.0	2.0 685.0
			26	0.0 0.0	0.0 0.0
			35	6.0 91.0	2.0 106.0
3	ROSS	Non	1	0.0 0.0	1412.5 1059.5
			8	0.0 0.0	16.0 318.0
			17	0.0 0.0	25.0 314.1
			26	0.0 0.0	16.0 318.0
			35	0.0 0.0	2871.1 925.7
4	ROSS	treated	1	0.0 0.0	1412.5 1059.5
			8	0.0 0.0	15.0 956.4
			17	0.0 0.0	0.0 0.0
			26- 35	0.0 0.0	0.0 0.0

**Table 2.** Results of SPA test for MG and MS in treated and non treated Cobb and Ross broiler chickens (n=40).

Age/ days	Treatment	Cobb broiler				Ross broiler			
		MG		MS		MG		MS	
		NO. of +ve	% of +ve	NO. of +ve	% of +ve	NO. of +ve	% of +ve	NO. of +ve	% of +ve
1	-	4	10	12	30	11	27.5	15	37.5
8		16	40	14	35	10	25	25	62.5
17		14	35	16	40	16	40	16	40
26		18	45	17	42.5	20	50	18	45
35		24	60	32	80	20	50	20	50
1	+	6	15	10	25	11	27.5	12	30
8		10	25	32	80	8	20	10	25
17		14	35	16	40	12	30	16	40
26		18	45	17	42.5	16	40	20	50
35		24	60	36	90	18	45	18	45

Generally; the livability taken in inconsideration the growth and mortality rates of treated flock was better than those of same non treated flocks breed (Levisohn and Kleven ,2000; Saif et al, 2003, Amer et al; 2009a , b and Zakeri and Kashefi, 2011).

Our results indicated that the used drug played a role in controlling infection (Saggiolato, et al, 2000 and Saif, et al, 2003) and limited gross lesions. These finding accord with results of Kempf, et al, (1997b); Charleston, et al. (1998); Guarini, et al (1999); Jordan and Horrocks, (1996) and Saggiolato, et al,(2000). Also; Mavromati, et al. (2011) found that Tilmicosin was used for mycoplasmas control in broilers flock were MG and MS positive (ELISA's test and Cultural control), where the results showed that the flock treated with Tilmicosin had a better control on mycoplasmas and better technical and economical results than the control flock. Talebi and Ghasemi-lak (2004) noted that the Tiamulin resolved the Mg and Ms infections and had the highest effect on both Mg and Ms ELISA titres, infected birds but do not completely cure the birds from the infections.

It can be concluded that usage of Tilmicosin can be essential in the treatment and prevention of CRD in broilers derived from positive breeders. In the other hand, the usage of such drug must be controlled to keep sensitivity of the organism as Gautier-Bouchardon et al. (2000) found that no resistance to Tiamulin could be evidenced in MG or MS after 10 passages.

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