

# **Comparative Therapeutic Efficacy of Different Drugs Against Caprine Contagious Agalactia**

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**Received:** 22 Aug., 2017

Revised: 09 Sept., 2017

Accepted: 26 Sept., 2017

#### ABSTRACT

The present work was undertaken with the aim to compare the therapeutic efficacy of different drugs against contagious agalactia in lactating goats. A total of 24 lactating goats having clinical mastitis and positive for *Mycoplasma agalactiae* were placed randomly into four groups i.e.  $T_1 - T_4$ , each group comprised of six mastitic goats and subjected to different therapeutic regimens. Six clinically healthy goats were kept as healthy control. *Mycoplasma agalactiae* was detected and confirmed as causative agent of contagious agalactia by using polymerase chain reactions which showed a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R. The response of therapeutic study was evaluated on the basis of clinical score card, clinical parameters and polymerase chain reactions on day 0 pretreatment and on days 7 and 14 post treatment. At the time of sampling, the clinical signs in sick animals were of clinical mastitis i.e. swelling of udder, abnormal milk secretion, pain on palpation of the udder and raised clinical parameters. The response of therapeutic study revealed significant improvement in temperature, pulse and respiration in all the treatment groups. On the basis of score card and polymerase chain reactions results on days 7 and 14 post-treatment, goats of the group  $T_4$  that was subjected to the treatment with combination of tylosin, tocopherol and sodium selenite showed earliest recovery.

Keywords: Contagious agalactia, mastitis, treatment, score card

Goats are frequently exposed to ravages of infectious diseases. Among various goat diseases, mycoplasmal infections are one of the important infections which result in significant losses (DaMassa *et al.*, 1992). The principal mycoplasma infection of small ruminants is contagious agalactia. Contagious agalactia affects all types of stock breeding, both traditional and intensive, throughout the world and its preferential mammary involvement presents a major health obstacle in the development of goat production (Bergonier *et al.*, 1997).

It is a disease predominantly of milking goats soon after lactation. The economic impact of the disease lies in the decrease or loss of milk production. In lactating female animals, it is usually manifested by mastitis (Madanat *et al.*, 2001) and mastitis along with decrease in milk production (Pooladgar *et al.*, 2011).

Mycoplasma, the causative agent of contagious agalactia, is inherently refractory to certain groups of antibiotics owing to its lack of cell wall. Antibiotics showing efficacy for Mycoplasma should possess the characteristics like activity against bacteria without cell wall, long persistence in the plasma, efficient diffusion into tissues, passage of high concentrations from the blood to milk (even becoming concentrated in the mammary gland) and very low minimum inhibitory concentrations (MIC).

Initial therapy for the infection included the use of arsenicals, particularly sodium and zinc salts of acetarsol. The continuous therapeutic usage of these compounds had adverse effects. However, the latest therapy for the disease is based solely on antibiotics like tetracycline, macrolide, tiamulin and fluoroquinolones. Although the significance of mycoplasmosis is well known but a meager work



regarding therapeutic management of caprine contagious agalactia has been carried out in Madhya Pradesh. Therefore, the present study was carried out to evolve a suitable therapy for caprine contagious agalactia.

# MATERIALS AND METHODS

#### Animals and sampling

The study was performed in 24 lactating goats naturally suffering from contagious agalactia due to *Mycoplasma agalactiae*. At the time of sampling, the clinical signs in sick animals were of clinical mastitis i.e. swelling of the udder, abnormal milk secretion, pain on palpation of the udder and raised clinical parameters.

Milk and mastitic secretions were collected aseptically from sick lactating goats for California mastitis test and identification of *Mycoplasma agalactiae* by polymerase chain reactions (PCR). Samples were collected on day 0 pretreatment and on days 7 and 14 post treatment.

## Design for therapeutic trial

To study the efficacy of different drugs for the treatment of contagious agalactia, a total of 24 lactating goats positive for *Mycoplasma agalactiae* were placed into four groups i.e.  $T_1 - T_4$ , each group comprised of 6 goats. Six clinically healthy goats were kept as healthy control (Group C). The details of therapeutic trial are presented in Table 1.

 Table 1: Design for therapeutic trial against contagious agalactia in goats

Group	Number of goats	Drugs and Dosage
T <sub>1</sub>	06	Oxytetracycline* @ 15 mg/kg b.wt., $I/M \times 5$ days
$T_2$	06	Tylosin @ 20 mg/kg b.wt., $I/M \times 5$ days
T <sub>3</sub>	06	Oxytetracycline* @ 15 mg/kg b.wt., I/M × 5 days + Tocopherol @ 2 mg/kg b.wt & Sodium selenite @ 0.06 mg/kg b.wt, I/M once
T <sub>4</sub>	06	Tylosin @ 20 mg/kg b.wt., I/M × 5 days + Tocopherol @ 2 mg/kg b.wt & Sodium selenite @ 0.06 mg/kg b.wt, I/M once
С	06	—

In addition to antimicrobial drugs, symptomatic and supportive therapy was done by administration of fluids and electrolytes (Inj. Normal saline @ 20 ml/kg body wt. IV, anti-inflammatory drugs (Inj. Meloxicam @ 0.3 mg/kg body wt. IM), antipyretic drugs (Injection Paracetamol @ 10 mg/kg body wt. IM) were given according to the clinical condition.

#### **Parameters of study**

The body temperature, pulse and respiration rates, California mastitis test and identification of *Mycoplasma agalactiae* by polymerase chain reactions were carried out on day 0 pretreatment and on days 7 and 14 post treatment.

#### **Analytical procedures**

Milk samples were used for performing the California mastitis Test and Polymerase chain reaction.

#### California mastitis test

The CMT was carried out in milk samples as described by Schalm *et al.* (1971) and interpretation of result was done according to Shearer and Harris (1992).

#### Identification of Mycoplasma agalactiae

Polymerase chain reaction (PCR) was performed to identify the *Mycoplasma agalactiae* and thus for confirmatory diagnosis of contagious agalactia. The DNA required for PCR was extracted from the milk samples of suspected animals. Approximately 0.1 ml of milk samples were inoculated into mycoplasma broth base containing mycoplasma supplement and incubated in a humid atmosphere containing 5% carbon dioxide at 37°C for 7 days. The broths were checked daily for growth. Positive broth cultures were stored at 4°C till further use (Kizil *et al.*, 2007).

DNA extraction was performed by chelex based extraction of DNA using Insta Gene Matrix (Bio-Rad laboratories, India Pvt. Ltd.) as described by Giraffa *et al.* (2000). The specific primers used were,

- ma-mp15'-AGCAGCACAAAACTCGAGA-3' (forward)
- ma-mp 1 5'-AACACCTGGATTGTTTGAGT-3' (reverse)

PCR products with a molecular size of 176 bp were considered indicative for Mycoplasma agalactiae.

#### Therapeutic response evaluation

The response of therapeutic study for contagious agalactia in lactating goats was evaluated on the basis of improvement in clinical parameters viz. temperature, respiration and pulse rates, score card and polymerase chain reactions after the completion of treatment i.e. on days 7 and 14 post treatment.

## **Score Card**

The efficacy of treatment in clinical mastitis can be judged by improvement in three categories viz. abnormal milk, abnormal gland and abnormal animal behavior (Radostits *et al.*, 2010). As mastitis is the outward clinical manifestation of contagious agalactia. Hence, keeping this view in mind, a score card was prepared for the first time on the basis of recovery in various parameters to assess the efficacy of various treatments in different groups. The parameters were selected as CMT for abnormal milk, inspection and palpation of udder for abnormal gland and behavior for abnormal animal scores were allotted as per the following table.

Davamatar	Score						
rarameter	0	1	2	3			
СМТ	No reaction	Distinct slime but without gel	Immediate gel formation	Gel develops a convex surface and adheres to surface			
Udder inflammation	None	Mild	Moderate	Severe			
Behavior	Alert	Sluggish	Lethargy	Apathy			

Each goat was observed for CMT reactions, udder inflammation and behavior. Score was given in each row representing the marks allotted to each parameter. Total score was calculated by adding the score of individual parameter and assessment was done as per the following score card.

Score	Interpretation
0	Healthy
1 – 3	Mild illness
4 - 6	Moderate illness
7 - 9	Severe illness

Table 2: Clinical Parameters in different treatment groups at different sampling intervals

	Treatmont	Sampling Intervals					
Parameters	Groups	Day 0	Day 7	Day 14			
	Groups	(Pre treatment)	(Post treatment)	(Post treatment)			
Temperature (°F)	T1	104.0 <sup>a</sup> ±0.18	102.3 <sup>bc</sup> ±0.46	102.0 <sup>bcd</sup> ±0.41			
	T2	103.5 <sup>a</sup> ±0.28	102.5 <sup>bc</sup> ±0.29	101.8 <sup>d</sup> ±0.29			
	Т3	103.7ª±0.23	102.3 <sup>bc</sup> ±0.36	$101.7^{d}\pm0.40$			
	Τ4	103.5ª±0.25	101.5 <sup>d</sup> ±0.25	101.2 <sup>d</sup> ±0.35			
	С	101.3 <sup>d</sup> ±0.24	101.3 <sup>d</sup> ±0.15	$101.4^{d}\pm0.09$			
Pulse rate	T1	94.7ª±0.88	75.8 <sup>b</sup> ±1.87	71.8 <sup>c</sup> ±1.60			
(per minute)	T2	93.5ª±0.76	73.0 <sup>b</sup> ±1.67	70.6°±0.88			
	Т3	94.0 <sup>a</sup> ±1.0	73.3 <sup>b</sup> ±2.25	69.6°±2.01			
	Τ4	93.0ª±1.09	69.8°±0.75	70.3°±0.76			
	С	71.5°±0.72	71.0°±0.85	71.3°±0.56			
Respiration rate (per	T1	47.8 <sup>a</sup> ±1.14	34.0 <sup>b</sup> ±2.20	30.5 <sup>bc</sup> ±1.86			
minute)	T2	47.8 <sup>a</sup> ±1.08	35.3 <sup>b</sup> ±2.47	31.0 <sup>bc</sup> ±1.98			
	Т3	45.5ª±1.30	35.5 <sup>b</sup> ±2.80	32.0 <sup>bc</sup> ±2.36			
	Τ4	45.3ª±1.75	27.1 <sup>cd</sup> ±1.42	26.8 <sup>cd</sup> ±1.37			
	С	24.1 <sup>d</sup> ±0.47	24.0 <sup>d</sup> ±0.45	24.5 <sup>d</sup> ±0.43			

Different superscripts indicate a significant difference (p≤0.05)

Journal of Animal Research: v.7 n.5, October 2017



#### Statistical analysis

The alterations in clinical parameters in different treatment groups at different intervals were analyzed using hierarchical design of ANOVA and means were compared using Duncan's multiple range test (Snedecor and Cochran, 1994).

# **RESULTS AND DISCUSSION**

## **Clinical parameters**

The detailed variation in clinical parameters viz. temperature, respiration rate and pulse rate in different treatment groups at different intervals are outlined in Table 2.

The results revealed that clinical parameters of affected goats on day 0 pre-treatment was significantly higher in all treatment groups as compared to healthy control group. A significant improvement in all the parameters were noticed in all treatment groups on days 7 and 14 post-treatment. However, the mean values of parameters on day 7 post treatment in group  $T_4$  were essentially similar to healthy goats.

The literature on the study of clinical parameters in contagious agalactia is meager. Although, Madanat et al. (2001) stated the presence of brief febrile syndrome due to mycoplasmaemia in infected animals. However, few scientists (Kizil et al., 2007 and Macun et al., 2010) reported transient fever, rise in pulse and respiration rate along with other symptoms in goats infected with Mycoplasma agalactiae and observed that after treatment these parameters were reduced to normal values. The results of present study are in correlation with that of previous workers. Raised clinical parameters in ailing animals are an indication of ongoing infection with other signs specific for contagious agalactia (Macun et al., 2010) and improvement after treatment showed the efficacy of drugs for that disease. However, treatment with tylosin along with tocopherol and sodium selenite in the animals of group  $T_A$  proved to be most efficacious in terms of improvement in clinical parameters. The results of present study were in accordance with those of previous studies (Bergonier et al., 1997 and Azevedo et al., 2006). There was a gradual improvement in terms of clinical picture of the disease and no death was reported in goats

during the study period. Similar drugs were used by Ball *et al.* (1987) for therapeutic trial in ewes experimentally infected with *Mycoplasma californicum* and reported that three days treatment with both oxytetracycline and tylosin successfully eliminated the infection. Moreover, some more drugs i.e. erythromycin, spiramycin, lincomycin and enrofloxacin along with tylosin were used by Loria *et al.* (2003) in the treatment of contagious agalactia and favourable results were obtained.

# **Clinical scores**

The details of clinical scores of individual goats under therapeutic trial in different groups are represented in Table 3.

 Table 3: Clinical score in goats at different intervals in different treatment groups

Group	Goats	Pre treatment	Post treatment (days)						
		Day 0	1	2	3	4	5	7	14
T <sub>1</sub>	i	08	08	07	06	03	01	00	00
	ii	09	09	08	06	05	03	02	00
	iii	09	09	08	07	05	04	03	01
	iv	07	07	06	04	03	01	00	00
	v	08	07	06	04	02	01	00	00
	vi	09	09	07	06	05	04	03	00
T <sub>2</sub>	i	08	08	06	04	02	00	00	00
	ii	09	09	08	06	04	02	00	00
	iii	08	08	07	06	05	03	00	00
	iv	08	07	06	05	02	00	00	00
	v	09	08	07	05	03	00	00	00
	vi	07	06	04	02	00	00	00	00
T <sub>3</sub>	i	09	09	08	06	05	02	00	00
	ii	09	09	08	06	05	03	02	00
	iii	06	06	05	03	02	00	00	00
	iv	08	08	07	05	03	01	00	00
	v	08	08	07	06	04	02	00	00
	vi	09	08	07	05	03	00	00	00
T <sub>4</sub>	i	08	08	05	04	02	00	00	00
	ii	09	08	07	05	03	00	00	00
	iii	06	06	05	03	00	00	00	00

i	v	07	07	05	02	00	00	00	00
١	7	08	08	07	05	03	00	00	00
v	i	09	08	06	04	02	00	00	00

In the present study, 23 goats were severely ill and two were moderately ill. After scheduled treatment, a gradual recovery was noticed in all the goats but fastest being in the goats of group  $T_4$ . The second most efficacious treatment group in terms of clinical recovery was group  $T_3$  followed by group  $T_2$  and group  $T_1$ .

Clinical score can be used as indicator of health. However, it is based on subjective judgment. Some of the scoring systems have been developed for other diseases but no traceable literature is available in regard to caprine contagious agalactia. As in the present work, the therapeutic study was conducted in confirmed cases of contagious agalactia in female lactating goats with mastitis as one of the clinical sign. So, for numerical assessment of the response of therapy against contagious agalactia in goats and on the basis of three categories stated by Radostits et al. (2010), a clinical score card was formulated for the first time. In the present study, gradual recovery was noticed in all the goats but fastest being in the goats of group  $T_4$  i.e. receiving treatment with tylosin along with tocopherol and sodium selenite. The second most efficacious treatment group in terms of clinical recovery was of goats receiving treatment with tylosin alone followed by goats receiving treatment with combination of oxytetracycline, tocopherol and sodium selenite. The treatment group  $T_1$  i.e. oxytetracycline alone was comparatively least efficacious.

#### Polymerase chain reaction

The growth of mycoplasmas in broth media was demonstrated by changes in colour or turbidity due to the bacterial biochemical activity and metabolism. When milk samples collected from 24 lactating goats suspected of contagious agalactia were subjected to PCR test at day 0 pre-treatment, a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R were observed.

After receiving treatment when the milk samples were again subjected to PCR on day 7 post treatment, two goats of group T1 showed the presence of organism, but on day 14 post treatment no organism was detected in PCR. This showed complete recovery in goats of group T, on day 14

post treatment. Similarly, in goats of group  $T_2$ ,  $T_3$  and  $T_4$ , PCR tests on days 7 and 14 post treatment revealed no specific bands indicating complete recovery from seventh day onwards in these groups.

In the present study, PCR was used for the confirmatory diagnosis of contagious agalactia. Use of PCR in the confirmatory diagnosis of contagious agalactia is supported by work of many scientists (Kizil et al., 2007; Zendulkova et al., 2007; Amores et al., 2010; Bidhendi et al., 2011 and Kheirkhah et al., 2011). In the present investigation, only PCR was adopted for identification of Mycoplasma agalactiae in milk samples because it has provided a rapid and early diagnosis when performed in clinical samples. Thus, it enabled to carry out early therapeutic measures when results were positive. Moreover, mycoplasmas are very fastidious pathogens (Srivastava et al., 2010) and isolation of mycoplasmas is considered to be one of the most difficult tasks for diagnostic laboratories due to their inability to grow easily in laboratory medium in spite of the great improvement in medium formulations (Al-Momani et al., 2006). PCR is a newer technique based on molecular biology. Mc Auliffe et al. (2003) stated that in near future molecular technology would be used not only to identify mycoplasma species, but also to detect them without the need of culture. PCR has the advantage of easy use, rapid availability of results and is more suited for processing large number of specimens (Kizil et al., 2007).

The therapeutic regimen in the present study included antibiotics like oxytetracycline and tylosin. Both proved to be efficacious in alleviating the mycoplasma infection. This might be due to the fact that mycoplasma the causative agent of contagious agalactia, lacks cell wall and thus is inherently refractory to certain groups of antibiotics. However, the mechanism of action of both tylosin and oxytetracycline is by inhibiting protein synthesis, so they work well for mycoplasmas. The higher dose rate of drugs and five day period of treatment in the present study was selected because of the previous lack of success of two days treatment (Mackie et al., 2000) and also due to the fact that results of treatment may be poor or ineffective against mycoplasmas if the therapeutic dose is not well defined and the antibiotics are not administered for a sufficiently long period (Madanat et al., 2001).

In the present investigation, tylosin with the combination of tocopherol and sodium selenite was found to be most



efficacious. Addition of antioxidants in the therapy might have increased the efficacy. The probable reason is that, many potentially toxic reactive oxygen species are generated through normal oxidative metabolism and the body has adapted by developing a complex system of protective antioxidants. Under more stressful conditions such as mastitis which is one of the most principal clinical symptoms in agalactia, hydroxy radicals are released by infiltrated neutrophils causing mammary cell injury and abnormal radical production leads to oxidative stress. Hence, it can be useful to add the antioxidant vitamins to the classical treatment procedures to get rid of the disease (Kizil *et al.*, 2007).

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