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Comparison of Marbofloxacin and Tylosin for treatment of goats affected with *Mycoplasma ovipneumoniae*#

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Abstract

Caprine respiratory mycoplasmosis is a major problem in India. Various mycoplasma species cause pneumonia and respiratory problems, thus inflicting huge economic losses in the small ruminant production sector. Though multiple species cause respiratory mycoplasmosis, data regarding species prevalence is deficient in India. The present study was conducted to study the prevalence of respiratory mycoplasmosis caused by Mycoplasma ovipneumoniae in goats using polymerase chain reaction (PCR). A total of 98 nasal swabs were collected, out of which 44 samples were positive for M. ovipneumoniae. A total of 26 animals with explicit clinical signs were selected for comparing the therapeutic efficacy of marbofloxacin and tylosin and were randomly divided into two groups consisting of 13 animals each. Group 1 was treated with marbofloxacin @ 2mg/kg body weight, intramuscularly for 5 days. Group 2 was treated with tylosin @ 10mg/kg body weight, intramuscularly for 5 days. Complete bacteriological recovery rate in group 1 and group 2 was observed as 69.2 per cent and 84.6 per cent respectively by day 11 of treatment. Statistical analysis revealed that marbofloxacin was as effective as tylosin against respiratory mycoplasmosis in goats.

Keywords: *PCR, M. ovipneumoniae, Marbofloxacin, Tylosin*

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Mycoplasmosis is one of the most challenging diseases of small ruminants, posing huge economic losses throughout the world. The economic losses result primarily because of emaciation, stunted growth, infertility and delayed market weight. *M. ovipneumoniae* is known to cause a non-progressive pneumonia in small ruminants. The organism is known for variability in strains and causes variation in patterns of mortality and morbidity. It has been suggested that *M ovipneumoniae* causes ciliostasis and facilitates the invasion of opportunists into the respiratory tract (Ongor *et al.,* 2011). Reji (2018) and Sumith (2021) reported the presence of *Mycoplasma ovipneumoniae* among the goat population of Wayanad district of Kerala. Santhiya *et al*. (2021) reported that 81.93 per cent were positive for *M. ovipneumoniae* specific PCR in northern and central parts of Kerala.

Culture and isolation of mycoplasmosis is a cumbersome process due to the fastidiousness of the organism and as it requires specialised laboratory facilites and costly media, and is hence not practiced everywhere. Serological tests are mostly employed in conjunction with culture, which has limitation of cross-reactivity with other *Mycoplasma* species. Thus, the value for molecular detection of mycoplasmosis is high. PCR assay being rapid, highly sensitive and specific, is recommended and considered a reliable tool for early detection of caprine respiratory disease (Manimaran *et al*., 2020).

Macrolides especially tylosin, are considered to be the drug of choice against contagious caprine plueropneumonia. Antibiotics like tetracyclines, fluoroquinolones and macrolides are commonly used against respiratory mycoplasmosis in goats (Ozdemir *et al*., 2006). Marbofloxacin, a new fluoroquinolone, has a wide spectrum of activity against *Mycoplasma* spp., gram-negative and gram-positive pathogens and has good penetration rate into the lung tissues (Balicki *et al*., 2008). The aim of the study was to compare the efficacy of marbofloxacin and tylosin in treating *M. ovipneumoniae* associated respiratory disease in goats.

Materials and methods

A total of 98 nasal swab samples were collected from goats exhibiting respiratory signs such as cough, nasal discharge, fever and abnormal respiratory sounds. Samples were collected from goats presented at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, Peripheral Veterinary Clinic, Kakkavayal and other goat farms in Wayanad, Kerala. The samples were collected using sterile nylon flocked swab with two breakpoints and were secured in screw cap vials containing phosphate buffered saline. The samples were then transported to laboratory under cold chain and subject to PCR for diagnosis. Detailed clinical examination was performed with special emphasis on respiratory system in presented goats. Clinical parameters such as temperature, heart rate and respiratory rate were observed and recorded.

Total DNA was extracted from nasal swabs using conventional Phenol-chloroform method. The purity and concentration of the extracted genomic DNA was estimated using Nanodrop 2000 UV-vis spectrophototmeter (Thermo Scientific, USA).

Detection of *M. ovipneumoniae* was done as per PCR protocol developed by Daee *et al*. (2020) using primers LMF1 (5'-TGAACGGAATATGTTAGCTT-3') and LMR1 (5'- GAGTTCATCCTGCACTCTGT-3'), targeting species specific region in the 16SrRNA gene. The reaction was carried out in a volume of 25 µL reaction mix which consisted of 12.5 µL of 2X EmeraldAmp PCR Master Mix containing Taq polymerase (TaKaRa), 1µL each of 10 pmol forward and reverse primers, 5 µL of DNA, and 5.5 µL NFW. The positive control used in the study was a field case DNA sample that tested positive for *M. ovipneumoniae*, while nuclease-free water was used as the negative control. The products were subjected to 1.5 per cent agarose gel electrophoresis and visualized with the gel documentation system. One of the positive samples was sequenced and confirmed as *M. ovipneumoniae* (NCBI Accession No. OQ910485).

In order to compare the efficacy of

the two antibacterial drugs, 26 PCR positive animals with overt clinical signs were selected and randomly divided into two groups. Group 1 was treated with marbofloxacin @ 2mg/kg body weight, intramuscularly for 5 days. Group 2 was given tylosin @ 10mg/kg body weight, intramuscularly for 5 days. Supportive therapy such as Flunixin meglumine @1.1mg/kg and Chlorpheniramine maleate@ 0.5mg/kg and vitamin B complex were given to both treatment groups for first five days. Nasal swabs were collected at 0^{th}, 5^{th}, 8^{th} and 11^{th} days of treatment and subjected to PCR. Treatment was continued up to 11 days if clinical signs persisted or PCR were found positive. Vital parameters and haematological parameters were recorded before and after treatment. Response to treatment was assessed based on the rapidity of clinical recovery, bacteriological recovery and changes in clinical and haematological parameters. Statistical analysis was done by independent t test between groups and paired t test within each group.

Results and discussion

In the present study, out of 98 samples, 44 (44.89 per cent) yielded amplicons at 361bp on PCR targeting *M. ovipneumoniae* gene indicating presence of the pathogen (Fig.1). BLAST analysis of sequencing results of amplicons obtained from *M. ovipneumoniae* specific PCR had 97.35 per cent identity to *M. ovipneumoniae.* Santhiya *et al*. (2021) reported the presence of *M. ovipneumoniae* in northern and central part of Kerala with a prevalence of 81.93 per cent. Sumith (2021) reported prevalence of 75.9 per cent of respiratory mycoplasmosis in Wayanad by genus specific PCR. The increased occurrence of respiratory mycoplasmosis in Wayanad might be due to the cold and humid environment prevailing in the region which predisposes to the disease condition.

All the goats treated for respiratory mycoplasmosis in the present study recovered clinically by day 11. On testing for bacteriological recovery, 23.1 per cent of Group 1 animals were tested PCR negative on day 5 of treatment and 69.2 per cent on day 11. A total of 46.15 per cent of animals in Group 2 recovered bacteriologically on day 5 and 84.6 per cent recovered by day 11. Paired t test revealed no significant difference in the recovery rate between group 1 and group 2, indicating marbofloxacin and tylosin to be equally effective in treating respiratory mycoplasmosis. Yatoo *et al.* (2019) reported rapid and faster recovery among tylosin treated group than oxytetratcycline followed by enrofloxacin treated group in Pashmina goats against CCPP. A recovery rate of 100 per cent was observed with the use of marbofloxacin at a dose rate of 2mg/kg for three days in CCPP

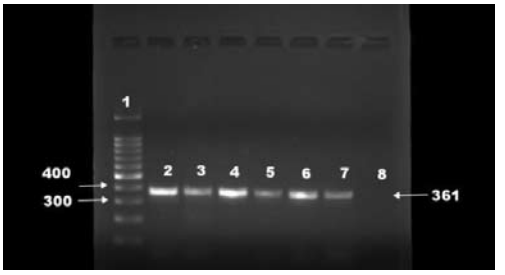

Fig. 1. Detection of *Mycoplasma ovipneumoniae*

(Lane 1: *100 bp ladder*, Lane 2: *Positive control*, **Lanes 3 to 7:** *Clinical samples*, **Lane 8:** *No template control*)

affected goats (Balikci *et al*., 2008).

Statistical analysis revealed no significant difference between group 1 and group 2 before treatment with respect to temperature, rate of respiration, total leukocyte count (TLC), granulocytes (G), lymphocytes (L), Monocytes (MON), total erythrocytes (TEC), haemoglobin (Hb) and volume of packed red cells (VPRC). However, heart rate was found to be significantly higher in group 2. Overall, the parameters indicated low variability between group 1 and group 2 (Table 1). Following treatment, significant reduction in heart rate and respiratory rate was recorded in both group 1 and group 2. Even though the rectal temperature decreased in both group post treatment, the decrease was not statistically significant in group 2. Akwuobu *et al*. (2014), Balicki *et al.* (2008) and Yatoo *et al.* (2019) reported no significant change in mean rectal temperature throughout the treatment period in cases treated for respiratory mycoplasmosis. The post treatment values were not significantly different between the groups.

Leukocytosis was evident among affected goats in the study. Significant reduction in total leukocyte, granulocyte, lymphocyte was observed in both group 1 and group 2. Reduction in monocyte count was significant in group 1 but not in group 2. Overall, the reduction in leukocyte counts post treatment indicated effective clearing of bacterial pathogens from the host system, and statistical insignificance between treatment groups indicated that both the drugs may be equally effective in clearing *M. ovipneumoniae* (Table 2). The findings are in contrast with Yatoo *et al*. (2019) who observed no changes in haematological parameters after

Table 1. Comparison of vital parameters in treatment groups

Parameter	Period	Group 1	Group 2	P value
Temperature (˚C)	Pre-treatment	39.26 ± 0.35	38.97 ± 0.35	0.306^{ns}
	Post-treatment	38.86 ± 0.20	38.82 ± 0.12	0.769^{ns}
	P-value	0.018*	0.326^{ns}	
Heart rate (beats/min)	Pre-treatment	91.15 ± 2.42	100.77 ± 3.65	0.038*
	Post treatment	83.92 ± 2.06	93.46 ± 2.28	0.005**
	P-value	0.002**	0.002**	
Respiratory rate (per min)	Pre-treatment	40.23 ± 1.55	41.54 ± 2.89	0.694^{ns}
	Post treatment	30.08 ± 1.24	31.54 ± 2.10	0.554^{ns}
	P-value	<0.001**	<0.001**	

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

Table 2. Comparison of haematological parameters in treatment groups

Parameter	Period	Group 1	Group 2	P value
TLC (10^3 cells/mm^3)	Pre-treatment	20.60 ± 1.33	19.13 ± 1.44	0.463^{ns}
	Post treatment	17.14 ± 1.38	14.81 ± 0.81	0.158^{ns}
	P-value	0.005**	< 0.001**	
GRAN (%)	Pre-treatment	49.25 ± 3.28	51.31 ± 3.03	0.650^{ns}
	Post treatment	43.95 ± 2.90	46.89 ± 1.87	0.402^{ns}
	P-value	0.004**	0.022*	
LYM (%)	Pre-treatment	46.35 ± 3.73	44.62 ± 2.98	0.720^{ns}
	Post treatment	50.38 ± 3.33	48.70 ± 1.68	0.657^{ns}
	P-value	0.048*	0.049*	
MON (%)	Pre-treatment	3.93 ± 0.26	3.92 ± 0.22	0.964^{ns}
	Post treatment	3.12 ± 0.15	3.67 ± 0.21	0.044*
	P-value	0.005**	0.474^{ns}	
TEC (10^5 cells/mm^3)	Pre-treatment	18.61 ± 0.64	17.34 ± 0.45	0.117^{ns}
	Post treatment	16.85 ± 0.55	16.08 ± 0.59	0.346^{ns}
	P-value	< 0.001**	0.028*	
HB (g/dL)	Pre-treatment	10.35 ± 0.39	10.61 ± 0.40	0.653^{ns}
	Post treatment	10.40 ± 1.46	8.59 ± 0.44	0.245^{ns}
	P-value	0.974^{ns}	0.002**	
VPRC (%)	Pre-treatment	23.20 ± 0.08	22.80 ± 0.06	0.711^{ns}
	Post treatment	22.30 ± 0.08	22.00 ± 0.08	0.857^{ns}
	P-value	0.003**	0.416^{ns}	

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

treatment but the findings in the current study may be due to good efficiency of the drugs used in the treatment trial. Statistical analysis revealed significant reduction in TEC post treatment in both groups. The VPRC values were found to be significantly reduced in group 1 but no significant change was reported in group 2. Haemoglobin values were found to be significantly lowered in group 2, but were not significantly different in group 1 Yatoo *et al.* (2019) reported favorable response and improvement in cardio-pulmonary function following treatment with tylosin in combination therapy.

Conclusion

In conclusion, the current study emphasises the incidence of respiratory mycoplasmosis in goats caused by *M. ovipneumoniae* in Wayanad, Kerala. The study supports the effectiveness of tylosin and marbofloxacin in treating respiratory mycoplasmosis in goats. Both medications reduced clinical symptoms and bacterial load and improved haematological and respiratory parameters in the afflicted animals equally well. In order to stop the spread of the illness and reduce financial losses in the goat farming business, early diagnosis and fast treatment of respiratory mycoplasmosis is warranted. Further research is needed to examine the development of antibiotic resistance in *M. ovipneumoniae* strains and to determine the long-term effects of therapy on the health and production of goats.

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Conflict of interest

The authors declare that they have no conflict of interest.

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