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Effect of different antimicrobials on oxidative indices in goats affected with caprine mycoplasmosis

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Abstract
Present study was conducted to study/explore the effect of various therapeutic regimes on the oxidative indices in mycoplasma affected goats. Thirty (N=30) clinically mycoplasma affected goats were taken and allotted to three test groups viz., Group I, II and III and were treated with different antimicrobials with each group having 10 animals (n=10). Group I was treated with tylosin @ 20 mg/kg, Group II with oxytetracycline @10 mg/, and Group III was treated levofloxacin @ 1.5 mg/kg body weight IM. Supportive medicines given with antimicrobials included flunexin meglumine @ 1.1 mg/kg and chlorpheneramine @ 0.5 mg/kg intramuscularly daily for three days in all test groups. Results indicated that at the 0th hour of the experimental trial, there were significant ($P \leq 0.05$) alterations in oxidative indices/values of all the three test groups compared to healthy control. With course of the trial, significant ($P \leq 0.05$) changes were observed in total oxidant status(TOS) and total anti-oxidant status (TAS) in group I alone indicating Tylosin gives better results compared to other antimicrobial agents.

Keywords: antimicrobials, caprine mycoplasmosis, goats, oxidative indices

Introduction
Mycoplasmosis occurs generally as a contagious disease in small ruminants and the infections are caused by various species of Mycoplasma microorganisms (Yatoo *et al*., 2018) [1]. They usually infect respiratory tract, reproductive tract, udder, eyes, joints and a few cause septicemia (Thiaucourt and Bolske, 1996; OIE, 2009) [2, 4]. Mycoplasmas are known to cause serious and often fatal illness in goats leasing to heavy morbidity (60-100%) and mortality (10-100%) in affected animals, resulting in severe loss to farmers (OIE, 2017; Yatoo *et al*., 2018) [5, 1]. The most commonly used as well as easily available antimicrobials till now are oxyteracycline, tylosin, enrofloxacin, tiamulin, danofloxacin (Sarkar *et al*., 1992; Kumar *et al*., 2012; Abraham *et al*., 2015) [3, 7, 10]. Antimicrobial use in combination with anti-inflammatory, anti- pyretic, analgesic and anti-allergic drugs under field conditions is uncommon but is essential for preventing the pathogen induced severe inflammation, fever, thorax pain (pleurodynia) and allergic reactions.
Excessive production of free radicals resulting in oxidative stress or oxidant/antioxidant imbalance occurs in diseased or stressful conditions (Nazifi *et al*., 2009) [11]. Irreversible damage to normal tissues might occur due to either excessive production or inadequate removal of free radicals (Lopaczyski and Zeisel, 2001) [12]. Altered levels of various oxidants and antioxidants have been reported in mycoplasma affected goats (Parray *et al*., 2019) [6]. Parray *et al*., 2019 [6] has reported decreased levels of TAS while increased levels of TOS in the mycoplasma affected goats.

Materials and Methods
Blood sampling
At the beginning of study, blood sample from each animal was collected by jugular puncture using sterile 18G needle. For estimation of oxidative indices (TAS, TOS and OSI) the blood was collected in clot activator (5ml) vials for serum extraction.

Procedure
The present study was conducted to explore oxidative changes in mycoplasma affected goats. Clinically affected goats (N=30) were divided in three groups *viz*. Group I, Group II and Group III with each group having minimum ten animals (n=10). An additional group with 10 healthy animals viz., Group IV was taken as healthy control or negative control. Group I, Group II and Group III were treated with tylosin @ 20 mg/kg, oxytetracycline @10 mg/kg and levofloxacin @ 1.5 mg/kg BW intramuscularly, respectively.

The treatment was repeated at the interval of 48 hours four times (0th hour, 48th hour, 96th hour, 144th hour). Supportive medicines given with antimicrobials included flunexin meglumine @ 1.1 mg/kg and chlorpheneramine @ 0.5 mg/kg intramuscularly daily for three days in all test groups. These groups were evaluated for oxidative indices at the beginning of the experimental trial viz., 0th hour and continued at the intervals of 48th hour, 96th hour, 144th hour. Improvement in oxidative indices were then used to aid in assessing the comparative efficacy of different therapeutic interventions. The therapeutic protocol is given in Table 1.

Total oxidant status (TOS) was evaluated as per the method of Erel (2004) [8] and was estimated through a new automated calorimetric method given by Erel (2004) [8]. The reading was taken at the wavelength of 560nm while the total antioxidant status (TAS) was estimated through a new direct automated method of Erel (2004) [8]. The reading was taken at the wavelength of 600 nm. It was followed by evaluation of oxidative stress index (OSI) which is the ratio of the total oxidant status (TOS) to total antioxidant status (TAS) (Erel, 2005) [9]. Oxidative stress index (OSI) was calculated as per the formula; OSI=TOS/TAS (Erel, 2005) [9]. Likewise, oxidative indices evaluation was also carried out in healthy control group.

Table 1: Therapeutic protocol

Group	Antibiotic (IM)	Supportive Treatment
Group I	Tylosin @ 20 mg/kg IM repeat 48 hourly	Flunexin meglumine @ 1.1 mg/kg and chlorpheneramine @ 0.5 mg/kg IM
Group II	Oxytetracycline @ 10 mg/kg repeat 48 hourly	Flunexin meglumine @ 1.1 mg/kg and chlorpheneramine @ 0.5 mg/kg IM
Group III	Levofloxacin @ 1.5 mg/kg repeat 48 hourly	Flunexin meglumine @ 1.1 mg/kg and chlorpheneramine @ 0.5 mg/kg IM
Group IV	NA	NA

Results and Discussion

Effect of different antibiotic based therapeutic regimens on oxidative indices

a) Total oxidative status (TOS) (µmol H_2O_2 equivalent/L)

At the beginning of the experimental trial, TOS levels were observed significantly ($P \leq 0.05$) higher in Group I, Group II as well in Group III compared tohealthy control/ negative control group. However, post-treatment, significant decrease ($P \leq 0.05$) was observed in Group I with values coming near close to healthy control at 144th hour of the therapeutic trial as shown in Table 2.

Table 2: Effect of different therapeutic regimes on TOS levels (µmol H_2O_2 equivalent/L)

Hours post treatment	Group I	Group II	Group III	Group IV
0	3.1480 ± 0.149^{a}	3.1660 ± 0.154^{a}	3.160 ± 0.161^{a}	1.5640 ± 0.068^{Aa}
48	2.6130 ± 0.131^{b}	2.8450 ± 0.144^{a}	2.890 ± 0.146^{a}	1.6120 ± 0.07^{Aa}
96	2.080 ± 0.114^{Ac}	2.5250 ± 0.14^{ABa}	2.6340 ± 0.133^{BCa}	1.5750 ± 0.073^{Da}
144	1.5590 ± 0.101^{Ad}	2.1990 ± 0.142^{Bb}	2.625 ± 0.134^{BCa}	1.5730 ± 0.069^{ADa}

^{ABCD}values with different superscript differ significantly ($P \leq 0.05$) in a row

^{abcd} values with different superscript differ significantly ($P \leq 0.05$) in a column

b) Effect on Total antioxidant status (TAS) (µmol Trolox equivalent/L)

In all three test groups the total antioxidant status (TAS) was significantly lower ($P \leq 0.05$) as compared to healthy control group (Group IV) at the beginning of the study. With commencement of treatment significant ($P \leq 0.05$) increase in Group I while non-significant ($P > 0.05$) increase in Group II and Group III was observed at all intervals as shown in Table 3.

Table 3: Effect of different therapeutic regimes on TAS levels (µmol Trolox equivalent/L)

Hours post treatment	Group I	Group II	Group III	Group IV
0	0.6257 ± 0.014^{a}	0.6228 ± 0.009^{a}	0.6193 ± 0.013^{a}	1.4650 ± 0.083^{Aa}
48	0.8235 ± 0.016^{b}	0.7205 ± 0.010^{a}	0.7067 ± 0.012^{a}	1.4610 ± 0.078^{Aa}
96	1.0091 ± 0.01^{Ac}	0.8240 ± 0.011^{Ba}	0.7883 ± 0.011^{BCa}	1.4670 ± 0.077^{Da}
144	1.1999 ± 0.016^{Ad}	0.9229 ± 0.011^{Bb}	0.8740 ± 0.014^{BCa}	1.4680 ± 0.074^{Da}

^{ABCD}values with different superscript differ significantly ($P \leq 0.05$) in a row

^{abcd} values different superscript differ significantly ($P \leq 0.05$) in a column

c) Effect on Oxidative stress index (OSI)

Pre-treatment at 0 hr, Group I, Group II and Group III showed significantly higher ($P \leq 0.05$) status in OSI compared to Group IV (healthy control). Post treatment significant ($P \leq 0.05$) decrease of OSI was observed in Group I and Group II of all the test groups. However, values for OSI in Group I were comparable to healthy control at 144th hour of the trial and percent decrease in OSI was more (74.2 %) in Group I as shown in Table 4.

Table 4: Effect of different therapeutic regimes on OSI

Hours post treatment	Group I	Group II	Group III	Group IV
0	5.018 ± 0.163^{a}	5.073 ± 0.207^{a}	5.136 ± 0.303^{a}	1.107 ± 0.087^{Aa}
48	3.163 ± 0.119^{Ab}	3.941 ± 0.172^{Bb}	4.110 ± 0.238^{BCb}	1.139 ± 0.087^{Da}
96	2.056 ± 0.096^{Ac}	3.058 ± 0.149^{Bc}	3.349 ± 0.179^{BCbc}	1.109 ± 0.09^{Da}
144	1.295 ± 0.075^{Ad}	2.375 ± 0.135^{Bd}	2.757 ± 0.158^{BCcd}	1.104 ± 0.08^{ADa}
% <(144thhr)	74.2	53.2	46.3	0

^{ABCD}values with different superscript differ significantly ($P \leq 0.05$) in a row

^{abcd} values with different superscript differ significantly ($P \leq 0.05$) in a column

In different test groups, from the experimental trial, improvement in oxidative indices post-treatment in different test groups was observed which indicate better and fast improvement in disease condition by action of antimicrobials combined with anti-inflammatory and anti-allergic agents (Yatoo *et al*., 2018) [1]. Tylosin showed significantly ($P \leq 0.05$) better results compared to other treatment agents used in the experimental trial in terms of improvement in oxidative indices. Effectiveness of antimicrobial drug tylosin has also been reported by Yatoo *et al*. (2018) [1].

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