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Correlation between anaplasmosis, anaemia and oxidative stress indices in goats of Thrissur, Kerala

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Abstract
Anaplasmosis is one among the most commonly encountered infectious diseases in goats, which causes considerable economic loss to farmers due to decreased milk yield, loss of body weight, abortion and eventually death of the animal. The study aimed to evaluate relationship between parasitemia due to *Anaplasma* spp., anaemia and oxidative stress markers in Thrissur district in Kerala state in the Indian subcontinent. Blood was collected from 16 female non-pregnant goats infected with *Anaplasma* species along with 8 healthy female animals as controls for evaluation of haematology and oxidative stress indicators. Percentage of parasitemia in diseased goats ranged from 1.5 to 7.2%. The values of total erythrocyte count (r = -0.94), haemoglobin (r = -0.97) and volume of packed red cells (r = -0.88) were decreased (p<0.01) whereas erythrocyte lipid peroxidation level (r = 0.84) was significantly (p<0.01) augmented in animals with parasitemia over control animals. In addition, levels of antioxidants such as superoxide dismutase (r = -0.87), catalase (r = -0.81) and reduced glutathione (r = -0.82) were also considerably reduced (p<0.01). Also, erythrocytic LPO was negatively correlated with erythrocyte count (r = -0.81), haemoglobin (r= -0.90) and packed cell volume (r = -.74) (p<0.01). Erythrocytic SOD was positively correlated (p<0.01) with TEC (r = 0.78), Hb (r = 0.87) and PCV (r = 0.69). GSH and catalase values were also positively correlated (p<0.01) with TEC, Hb and PCV. From the current study, it can be suggested that anaplasmosis in goats is associated with parasitic load associated oxidative stress as indicated by poor antioxidant status and enhanced pro oxidants, which are contributed to severe anaemia.

Keywords: Anaplasmosis, lipid peroxidation, antioxidants, oxidative stress, anaemia

1. Introduction
Goat farming can be a great source of income to the marginal and landless farmers in Kerala. Among all the livestock animals goats can be raised and managed easily than others. Anaplasmosis is one of the major haemoprotozoan diseases of ruminants caused by intraerythrocytic parasites of the order Rickettsiales [1]. The disease is characterized by pyrexia, anaemia, jaundice, lethargy, dairrhoea, dyspnoea, reduced milk yield and eventually death [2]. Severe infection with *Anaplasma ovis* even leads to reduced fertility and abortion [3]. *Anaplasma* infections lead to extravascular haemolytic anaemia and factors like phagocytosis of erythrocytes by reticulo-endothelial cells, oxidative damage, a high oxidative stress and immune mediated destruction of red blood cells contribute to anaemia [4].
Oxidative stress is described as an imbalance between oxidants and antioxidants level [5]. Variation of oxidative stress indices have been noticed in various parasitic diseases [6, 7]. Malondialdehyde (MDA), a product of lipid peroxidation can be used as an ideal indicator of oxidative stress [8]. Erythrocytic peroxidation plays an important role in the pathogenesis of haemoparasitic infestations [9, 10]. Superoxide dismutase, catalase and reduced glutathione are the antioxidants that are taken for the present study. The levels of these antioxidants varies in various parasitic infestations [11, 12]. Increased concentration of malondialdehyde and erythrocyte infection rate by *Theileria* species were positively correlated by various authors [13, 14]. *Anaplasma marginale* infection in calves was found to cause an increase in LPO level and decrease in SOD, catalase and GSH-Px [12]. The present study therefore intend to correlate parasitemia caused by caprine anaplasmosis to oxidative stress indices and anaemia.

2. Materials and Methods
Goats presented with clinical signs suggestive of anaplasmosis such as pyrexia, pale mucous membranes, jaundice, lethargy, anorexia and tick infestation were included in the study.

Female non-pregnant goats aged 1–3 years formed the experimental groups. Simultaneously, another 6 animals of same age group with normal physiological status and negative for *Anaplasma* infection (based on blood smear examination and polymerase chain reaction) were selected to serve as control group (group II).

2.1 Parasitological study
For parasitological examination, thin peripheral blood smears were prepared from clinically suspected animals, air-dried, fixed in methanol and stained with Field's stain and examined microscopically. Confirmation of the disease was done using polymerase chain reaction.

2.2 Quantification of parasitemia
To quantify the intensity of parasitemia, number of parasitized erythrocytes present per 1000 red blood cells was counted, then divided by 10 and expressed in percentage.

2.3 Collection of clinical samples
Two ml blood was collected from jugular vein from each goat in EDTA vials. Haemoglobin (Hb g/dL), volume of packed red cells (VPRC %) and total erythrocyte count (TEC x 10^6/µL) were analyzed for haematology. Four ml of blood was collected from jugular vein from each goat in heparin vials. Immediately after collection, blood samples were centrifuged at 2,000 rpm for 5 minutes in refrigerated condition. After aspirating plasma and buffy coat, the packed erythrocytes was resuspended in isotonic phosphate buffer saline (PBS, pH 7.4) and was centrifuged again at 5,000 rpm for 5 min, and the supernatants were discarded. This process was repeated two times. Finally, 1:20 dilution of erythrocyte haemolysate was prepared in triple distilled water for measurement and analysis of oxidative stress indices.

2.4 Estimation of oxidative stress parameters
Level of lipid peroxides in erythrocyte haemolysate was determined by estimating malondialdehyde (MDA) level [15]. Superoxide dismutase (SOD) in haemolysate was measured using nitro blur tetrazolium as substrate [16]. Catalase activity in erythrocyte haemolysate was determined photometrically [17]. Reduced glutathione activity was determined colorimetrically [18].

2.5 Statistical analysis
The statistical analysis of data obtained was done using computer software Statistical Package for Social Sciences (SPSS), version 24.0. The results were reported as means ± standard error (SE) for the infected and healthy group of animals. Data was analyzed statistically using paired *t* test. Pearson's correlation (r) was analyzed on paired data obtained by individual animals. $p<0.01$ was considered statistically significant.

3. Results
The examination of peripheral blood smears prepared from diseased animals revealed presence of *Anaplasma* spp. and parasitemia represented by percentage of *Anaplasma* infected erythrocytes ranged from 1.5 per cent to 7.2 per cent with a mean of 5.24±1.77 per cent. Correlation among parasitemia, haematological and oxidative stress parameters are depicted in Table1. The Pearson's correlation (r) of paired data of individual infected goats indicated significant ($p<0.01$) negative correlations between parasitemia and Hb (r = -0.978), TEC (r = -0.941) and PCV (r = -0.889). The mean value of erythrocytic LPO was significantly ($p<0.01$) raised and erythrocytic SOD, GSH and catalase levels were significantly ($p<0.01$) reduced in *Anaplasma* affected goats. In addition, percentages of parasitemia in infected goats were positively correlated ($p<0.01$) with LPO (r = 0.899) and negatively correlated ($p<0.01$) with SOD (r = -0.876), GSH (r = -0.825) and catalase (r = -0.810). Moreover erythrocytic LPO were negatively correlated ($p<0.01$) with TEC (r = -0.816), Hb (r = -0.909), and PCV (r = -0.746), whereas erythrocytic SOD was positively correlated ($p<0.01$) with TEC (r = 0.787), Hb (r = 0.873) and PCV (r = 0.693). Reduced glutathione (GSH) and catalase values were also positively correlated ($p<0.01$) with TEC, Hb and PCV.

Table 1: Correlation among parasitemia, haematological and oxidative stress parameters in diseased animals

		Parasitemia	RBC	Hb	VPRC	LPO	SOD	CAT	GSH
Parasitemia	Pearson correlation	1	-0.94**	-0.97**	-0.88**	0.89**	-0.87**	-0.82**	-0.81**
	p-value		0.000	0.000	0.000	0.000	0.000	0.000	0.000
RBC	Pearson correlation	-0.94**	1	0.94**	0.94**	-0.81**	0.78**	0.71**	0.68**
	p-value	0.000		0.000	0.000	0.000	0.000	0.002	0.003
Hb	Pearson correlation	-0.97**	0.94**	1	0.92**	-0.90**	0.87**	0.83**	0.79**
	p-value	0.000	0.000		0.000	0.000	0.000	0.000	0.000
VPRC	Pearson correlation	-0.88**	0.94**	0.92**	1	-0.74**	0.69**	0.63**	0.55*
	p-value	0.000	0.000	0.000		0.001	0.003	0.008	0.027
LPO	Pearson correlation	0.89**	-0.81**	-0.90**	-0.74**	1	-0.94**	-0.93**	-0.89**
	p-value	0.000	0.000	0.000	0.001		0.000	0.000	0.000
SOD	Pearson correlation	-0.87**	0.78**	0.87**	0.69**	-0.94**	1	0.93**	0.92**
	p-value	0.000	0.000	0.000	0.003	0.000		0.000	0.000
CAT	Pearson correlation	-0.82**	0.71**	0.83**	0.63**	-0.93**	0.93**	1	0.91**
	p-value	0.000	0.002	0.000	0.008	0.000	0.000		0.000
GSH	Pearson correlation	-0.81**	0.68**	0.79**	0.55*	-0.89	0.92**	0.91**	1
	p-value	0.000	0.003	0.000	0.027	0.000	0.000	0.000	
** - Significant at 1% level ($p<0.01$), * - Significant at 5% level ($p<0.05$)									

Table 2: Comparison of haematological parameters and oxidative stress markers in *Anaplasma* affected goats and healthy goats

Haematological parameters	Treatment groups		t-value	p-value
	Control (n=6)	Diseased (n=16)		
Total Leukocyte Count (10^3/µL)	8.06±0.63	7.65±0.41	0.543^{ns}	0.593
Lymphocytes (Per cent)	57.96±1.27	61.81±1.36	1.611^{ns}	0.123
Monocytes (Per cent)	4.70±0.18	4.58±0.31	0.307^{ns}	0.762
Granulocytes (Per cent)	37.33±1.25	33.59±1.23	1.716^{ns}	0.102
Total Erythrocyte Count (10^6/µL)	9.49±0.13	4.91±0.22	11.955**	0.000
Haemoglobin (g/dL)	8.41±0.09	5.59±0.44	3.855**	0.001
VPRC (Per cent)	36.06±0.76	17.38±0.84	12.649**	0.000
Platelet Count (10^5/µL)	4.66±0.50	4.21±0.44	0.569^{ns}	0.576
** - Significant at 1% level (p<0.01), ^{ns} – Non significant				

Oxidative stress parameters	Treatment groups		t-value	p-value
	Control (n=6)	Diseased (n=16)		
Lipid peroxidation (nanomoles MDA/mL)	3.56±0.13	6.43±0.10	14.789**	0.000
Superoxide dismutase (U/mg Hb)	2.16±0.10	1.30±0.06	7.009**	0.000
Catalase (U/mg Hb)	11.61±0.34	6.41±0.32	9.007**	0.000
Reduced glutathione (µmol/L)	2.95±0.24	1.74±.08	5.989**	0.000

** - Significant at 1% level (p<0.01)

4. Discussion

An imperative look to the result of correlation study disclose a positive correlation btween parasitemia and erythrocytic LPO, whereas negative correlation between parasitemia and SOD, catalase and GSH were revealed. Haematological parameters such as TEC, Hb and VPRC also revealed a negative correlation between parasitemia as well as erythrocytic LPO level. Infection with *Anaplasma* spp. stimulates the production of various pro-inflammatory cytokoines from mononuclear cells. These cytokines enhance the release of reactive oxygen species by phagocytic cells [19] which further activates lipid peroxidation. Polyunsaturated fatty acids, the most vulnerable substrate of lipid peroxidation is abundant in the membrane of erythrocytes [20]. Malondialdehyde, an important product of lipid peroxidation reacts with elements of cell membrane and thereby leads to increased permeability of cells and enzyme activities and ultimately lead to destruction of erythrocytes. Haemoglobin present in erythrocytes also acts as a potent promoter of oxidative processes. The result suggest that infection with *Anaplasma* spp. has a significant influence on enhanced LPO production and excess free radicals lead to a decreased antioxidant status. Hence, it could be concluded that severity of anaplasmosis is directly proportional to oxidative stress and depletion of antioxidant depot in body. Increased erythrocytic LPO level and decreased antioxidant activity have been reported in *Babesia bigemina*, *Theileria annulata* and *Anaplasma marginale* infections in cattle by different authors [21, 22]. In addition, negative correlation between LPO and anaemia and positive correlation between SOD, catalase and anaemia indicates that LPO plays an important role for development of anaemia and that antioxidants act in favour of erythrocytes to reduce oxidative stress.

5. Conclusion

The current study provides an association with parasitemia caused by Anaplasma spp. and oxidative stress. Hence inclusion of antioxidants in the treatment schedule of anaplasmosis will be helpful for faster recovery.

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