RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.4.703-708

Assessment of oxidative stress and antioxidant status in cattle infected with *Theileria orientalis*#

Lakshmi Mohanan[1*], S. Ajithkumar[2], Usha Narayana Pillai[3], Arun George[4] and Varuna P. Panicker[5]

Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, Kerala Veterinary and Animal Sciences University, Pookode-673 576, Wayanad, Kerala, India

Citation: Lakshmi,M., Ajithkumar,S., Usha,N.P., Arun,G. and Varuna,P.P. 2022.Assessment of oxidative stress and antioxidant status in cattle infected with Theileria orientalis. *J. Vet. Anim. Sci.* **53**(4): 703-708
DOI: https://doi.org/10.51966/jvas.2022.53.4.703-708

Received: 28.06.2022 *Accepted: 12.09.2022* *Published: 31.12.2022*

Abstract

Oriental theileriosis is a major haemoprotozoan disease of cattle and causes huge economic losses to the farmers. Oxidative stress plays an important role in the development of anaemia and subsequent complications associated with theileriosis. The present study was conducted to assess oxidative stress and antioxidant status of cattle infected with Theileria orientalis. In the present study, 16 animals which were positive for theileriosis by blood smear examination were selected. Confirmation of oriental theileriosis was done with polymerase chain reaction (PCR). Assessment of oxidative stress and antioxidant status were done by measuring malondialdehyde (MDA) levels and reduced glutathione activity respectively.

Both MDA levels and reduced glutathione activity did not indicate any significant difference in animals infected with T. orientalis from control animals.

Keywords: *Antioxidant status*, *cattle, oriental theileriosis, oxidative stress*

In India, bovine theileriosis is considered to be one of the most common and economically important haemoparasitic diseases of cattle. Theileriosis is a tick transmitted disease caused by haemoprotozoan parasites of the genus *Theileria* belonging to the phylum apicomplexa. Bovidae

 # Part of MVSC thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. *MVSc Scholar*
2. *Professor*
3. *Professor and Head*
4. *Assistant Professor*
5. *Assistant Professor, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy*

**Corresponding author: lakshmileela333@gmail.com, Ph: 8592031120*

are known to be infected by six different species of *Theileria*, among them *Theileria parva* and *Theileria annulata* are known as transforming *Theileria spp* (Von Schubert *et al*., 2010). *Theileria orientalis* produce non-transforming or benign theileriosis, which induce their pathologic effect through the destruction of erythrocytes (Eamens *et al*., 2013). Previously *T. orientalis,* causing oriental theileriosis was considered as a benign parasite. But recently there are increasing reports of fatal forms of the disease from various parts of the world (Izzo *et al*., 2010; Aparna *et al*., 2011; Vinodkumar *et al*., 2016).

Oxidative stress in veterinary medicine, especially in ruminant health is an emerging field of research and it is defined as an imbalance between oxidants and reductants at cellular level (Lykkesfeldt and Svendsen, 2007). Oxidative stress played an important role in the development of anaemia and subsequent complications associated with theileriosis (Grewal *et al*., 2005). Increased levels of erythrocytic lipid peroxidation in theileriosis are an indication of oxidative stress during the disease. Oxidative damage to RBCs may play a role in the aetiology of anaemia in bovine theileriosis (Shiono *et al*., 2003). Malondialdehyde (MDA), an end product of the oxygenation of polyunsaturated fatty acids, is a reliable and widely used biomarker for lipid peroxidation (Moore and Roberts, 1998). Decreased serum levels of antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH Px) and catalase in cattle with theileriosis is an antioxidant defence mechanism which enhance the reduction of free radicals (Hassanpour *et al*., 2013).

The goal of the current study was to determine and understand the antioxidant status and oxidative stress of cattle in oriental theileriosis.

Materials and methods

Cross bred dairy cattle which are apparently healthy and some with mild clinical signs such as reduced milk production, dullness, excessive salivation and anorexia in various farms of Thrissur district of Kerala were included in the study. Among these 16 animals which were positive for theileriosis in both blood smear and PCR were taken as diseased animals. Eight apparently healthy animals which were negative on both blood smear and PCR for theileriosis were taken as control animals. Cows between 3-6 years age formed experimental groups. Thin peripheral blood smears were prepared, air dried, fixed with methanol and stained by Fields stain (Methylene blue and eosin). DNA extracted from blood was subjected to species specific PCR using primers suggested by Ota *et al*. (2009). Species specific primers p23-F (5'-GTACACACCTTGAAATCTGGC-3') and p23-R (5'-CAAGAGAGGCAACAACAACGA-3') was used to amplify a 601 bp fragment of p23 gene of *T. orientalis*. The thermal cycling conditions included an initial denaturation at 94□ for 10 min, followed by 35 cycles of denaturation at 94□ for 1 min, annealing at 58.3□ for 1 min and extension at 72□ for 1 min and a final extension step for 5 min at 72 □.

Preparation of erythrocyte haemolysate

Heparinised blood samples collected in 4 ml sterile vials were centrifuged at 2,000 rpm for five minutes in refrigerated condition. After aspirating plasma and buffy coat, the packed erythrocytes were resuspended in isotonic phosphate buffer saline (PBS, pH 7.4), centrifuged again at 5,000 rpm for five minutes and the supernatant was discarded. This process was repeated two times. Finally, 1:20 dilution of erythrocyte haemolysate was prepared in triple distilled water. This erythrocyte haemolysate was used for assessing oxidative stress and antioxidant status.

Level of erythrocytic lipid peroxidation was determined by using the method of Placer *et al.* (1966) by estimating malondialdehyde (MDA) level. Reduced glutathione activity was determined by spectrophotometry by using reduced glutathione (GSH) assay kit supplied by Origin Diagnostics and Research, Kollam.

Haematological analysis

Haematological analysis was done using samples collected in EDTA coated vials. An automatic haematology analyzer (Orphee, Mythic Vet 18) was used to assess total

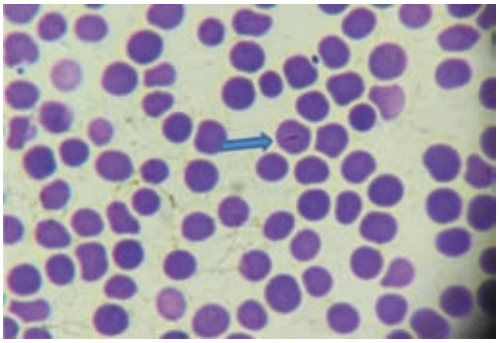
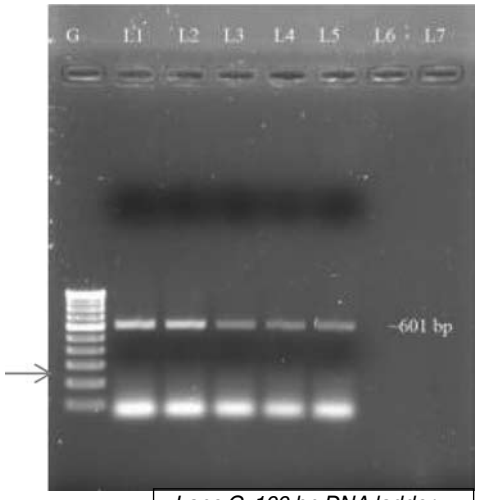


Fig. 1. Fields stained blood smear with rod shaped *Theileria orientalis* organism in an erythrocyte



Lane G: 100 bp DNA ladder
Lanes1-5 : Positive samples

Fig. 2. Agarose gel (1.5 %) electrophoresis of PCR products of *Theileria orientalis*

erythrocyte count (TEC), haemoglobin, volume of packed red cells (VPRC), total leucocyte count (TLC), differential leucocyte count (DLC) and platelet count.

Statistical analysis

The statistical analysis of data was done using computer software Statistical Package for Social Sciences (SPSS), version 24.0. Independent 't' test was used for the comparison between groups.

Results and discussion

On examination of peripheral blood smears, piroplasms of *Theileria* spp were observed in variable morphology such as thin and thick rods with trailing cytoplasm in all the 16 cattle selected for the study (Fig. 1).

The DNA extracted from blood samples collected in sterile EDTA coated vials of all the infected cows was subjected to species specific PCR. The species specific PCR with *p23*F and *p23* R primers yielded amplicons of size 601 bp specific for *T. orientalis* (Fig. 2).

Haematological parameters

The haematological parameters (Table 2) showed a statistically significant reduction in the mean values of TEC, haemoglobin and VPRC in *T. orientalis* infected animals compared with animals of control group. Similar findings were also reported by Izzo *et al.* (2010), Aparna *et al*. (2011), Jackson (2018) and Goud *et al*. (2021). This reduction in TEC, haemoglobin and VPRC could be due to the development of anaemia in these animals. In oriental theileriosis, pathogenesis of anaemia was not clearly understood and it may be variable. Lowered survival rate of erythrocytes (Yagi *et al*. 1991), lipid peroxidation and oxidative injury of the erythrocytes (Yagi *et al*. 2002) are proposed as the mechanisms resulting in extravascular haemolysis and subsequent anaemia in oriental theileriosis.

A significant increase was noted in the mean values of TLC and monocytes in infected animals. Mean values of platelet, granulocytes and lymphocytes showed no change in the study. Similar findings were also reported by Sudhakar (2020), who reported marked leucocytosis with lymphocytosis in animals infected with *T.orientalis.* Jackson (2018) reported normal values of WBC with increased levels of granulocytes and monocytes in oriental theileriosis. Aparna *et al*. (2011) observed leukopenia with normal DLC in animals infected with *T.orientalis*. But Izzo *et al*. (2010) reported that the changes in white blood cells were heterogeneous; some animals had normal leucocyte counts while others exhibited leucocytosis with neutrophilia and left shift.

Oxidative stress and antioxidant status

Malondialdehyde is the result of lipid peroxidation and its levels are employed

Table 1. Haematological profile of healthy and *Theileria* affected cows

Haematological parameters	Treatment groups		t-value	p-value
	Control (n=8)	Diseased (n=16)		
Total Leukocyte Count (10^3/µL)	7.025±0.49	9.36±0.5	2.94**	0.008
Lymphocytes (Per cent)	61.23±2.22	62.39±2.14	0.33ns	0.74
Monocytes (Per cent)	5.46±0.26	7.32±0.49	3.33**	0.003
Granulocytes (Per cent)	32.11±2.75	30.90±2.08	0.34ns	0.73
Total Erythrocyte Count (10^6/µL)	6.37±0.13	5.48±0.22	2.63*	0.015
Haemoglobin (g/dL)	8.07±0.17	6.75±0.22	3.83**	0.001
VPRC (Per cent)	30.21±1.44	25.77±0.78	2.94**	0.007
Platelet Count (10^5/µL)	279.38±32.03	337.13±25.33	1.36ns	0.187

** - Significant at 1 % level ($p < 0.01$), *- Significant at 5 % level ($p < 0.05$) ns – Non significant

Table 2. Oxidative stress parameters of healthy and diseased animals.

Oxidative stress parameters	Treatment groups		t-value	p-value
	Control (n=8)	Diseased (n=16)		
Malondialdehyde (nanomoles MDA/mL)	11.10±1.65	11.03±0.94	0.038ns	0.97
Reduced glutathione (µmol/L)	3.71±0.82	5.29±0.65	1.44ns	0.16

ns – Non significant

as an indicator of oxidative breakdown of polyenic fatty acids in the membranes as well as an indirect biomarker of free radical formation (Cimen, 2008). The oxidative stress and antioxidant status in cows infected with *T. orientalis* and control animals are given in the table 3. Statistically no significant differences were noticed in MDA values between infected and control animals. This result was in contrary to the findings of Shiono *et al*. (2003), Grewal *et al.* (2005), Razavi *et al*. (2012) and Nayak *et al*. (2018), where authors reported that significant elevation of MDA in theileriosis was an indication of oxidative stress.

However, Sahoo *et al*. (2001) reported that the rise in lipid peroxidation during theileriosis was insignificant. Saleh *et al*. (2011) also suggested that oxidative damage in *T. annulata* infection was dependent on parasitic burden. Mild clinical signs and lower levels of parasitaemia in the animals of present study might be the reason for nonsignificant changes in MDA in infected and non-infected animals.

Reduced glutathione (GSH) is a major cellular antioxidant defence mechanism as it acts as an essential cofactor for antioxidant enzymes such as glutathione peroxidase (GSH-Px), glutathione reductase and glutathione-S-transferase. Reduced glutathione is necessary for the GSH-Px-catalyzed removal of H_2O_2 from erythrocytes. This reaction is significant because an increase in the rate of oxidation of haemoglobin to methemoglobin may reduce the lifespan of erythrocytes (Razavi *et al*., 2011).

In the present study no significant difference was noted in reduced glutathione activity in cows affected with *T. orientalis* from control animals. This was against the findings of Razavi *et al*. (2012), where the authors reported reduced activities of the erythrocyte antioxidant enzymes superoxide dismutase, GSH-Px, and catalase in *T. annulata* infected cows when compared to non-infected controls. This indicated an extensive utilization and reduced production of endogenous GSH. Similar result was obtained by Nazifi *et al*. (2011) in a study conducted in tropical theileriosis in cattle. Grewal *et al*. (2005) reported a significant increase in GSX-Px activity in cows affected with *T. annulata* and the authors suggested that it can be attributed to an increased endogenous production of GSH in response to elevated oxidative stress.

Conclusion

In the present study, among 16 animals with oriental theileriosis, no significant difference was noted in both malondialdehyde

levels and reduced glutathione activity. Thus, it can be concluded that oxidative stress and antioxidant status did not indicate any appreciable deviations in animals infected with *T. orientalis* from normal animals.

Acknowledgement

The authors are grateful to Kerala Veterinary and Animal Sciences University for providing the research facilities to complete the study.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aparna, M., Ravindran, R., Vimal kumar, M.B., Lakshmanan, B., Ramesh kumar, P., Kumar, K.A., Promod, K., Ajith kumar, S., Ravi shankar, C., Devada, K. and Subramanian, H. 2011. Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India. *Parasitol. Int.* **60**: 524-29.

Cimen, M.B. 2008. Free radical metabolism in human erythrocytes. *Clin. Chim. Acta.* **390**: 1-11.

Eamens, G.J., Gonsalves, J.R., Jenkins, C., Collins, D. and Bailey, G. 2013. *Theileria orientalis* MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. *Vet. Parasitol.* **191**: 209-217.

Goud, K.S., Vijayakumar, K. and Davis, K.J. 2021. Haemato-biochemical studies of *Theileria orientalis* infection in cross bred dairy cattle. *J. Vet. Anim. Sci.* **52***:* 42-47

Grewal, A., Ahuja, C.S., Singha, S.P.S. and Chaudhary, K.C. 2005. Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of crossbred cattle naturally infected with *Theileria annulata*. *Vet. Res. Commun.* **29**: 387-394.

Hassanpour, A., Sabegh, Y.G., Sadeghi-nasab, A. and Hassanpour, A. 2013. Assessment of serum antioxidant enzymes activity in cattle suffering from Theileriosis. *Eur. J. Exp. Biol.* **3**: 493-496.

Izzo, M.M., Poe, I., Horadagoda, N., De Vos, A.J. and House, J.K. 2010. Haemolytic anaemia in cattle in NSW associated with Theileria infections. *Aust. Vet. J.*, **88**: 45-51.

Jackson, B. 2018. Clinico-therapeutic studies on bovine theileriosis. M.V.Sc thesis, Kerala Veterinary and Animal Sciences University, Pookode, pp, 123.

Lykkesfeldt, J. and Svendsen, O. 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. *Vet. J.* **173**: 502-511.

Moore, K. and Roberts, L.J. 1998. Measurement of lipid peroxidation. *Free radic Res.* **28**: 659-671.

Nayak, S.M., Senapati, S.K., Samal, P., Sethy, K., Meher, S., Swain, P., Sahoo, P.R. and Das, M.R. 2018. Therapeutic management of oxidative stress in cattle, naturally affected with bovine tropical theileriosis by vitamin e and selenium. *Pharm. Innov.***7**: 1141-1145.

Nazifi, S., Razavi, S.M., Hasanshahi, F. and Esmailnezhad, Z. 2011. Effect of the severity of *Theileria annulata* infection on some haematological parameters and antioxidant enzymes in naturally infected cattle. *Bulg. J. Vet. Med*. **12***:* 63-71.

Ota, N., Mizuno, D., Kuboki, N., Igarashi, I., Nakamura, Y., Yamashina, H., Hanzaike, T., Fujii, K., Onoe, S., Hata, H. and Kondo, S. 2009. Epidemiological survey of *Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan. *J. Vet. Med. Sci.* **71**: 937-944.

Placer, Z.A., Cushman, L.L. and Johnson, B.C. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal. Biochem*. **16***:* 359-364.

Razavi, S.M., Nazifi, S., Bateni, M. and Rakhshandehroo, E. 2011. Alterations of erythrocyte antioxidant mechanisms: Antioxidant enzymes, lipid peroxidation and serum trace elements associated with anemia in bovine tropical theileriosis. *Vet. Parasitol.* **180**: 209-214.

Razavi, S.M., Nazifi, S., Rakhshandehroo, E., Firoozi, P. and Farsandaj, M. 2012. Erythrocyte antioxidant systems, lipid peroxidation and circulating lipid profiles in cattle naturally infected with *Theileria annulata*. *Rev. Med. Vet.* **163**: 18-24.

Sahoo, A., Patra, R.C., Pathak, N.N., Dwivedi, S.K. and Dash, P.K. 2001. Enhanced lipid peroxide levels in the erythrocytes of calves with haemoglobinuria. *Vet. Res. Commun.* **25**: 55-59.

Saleh, M.A., Mahran, O.M. and Al-Salahy, M.B. 2011. Corpuscular oxidation in newborn crossbred calves naturally infected with *Theileria annulata*. *Vet. Parasitol.* **182**: 193-200.

Shiono, H., Yagi, Y., Chikayama, Y., Miyazaki, S. and Nakamura, I. 2003. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with *Theileria sergenti*. *Parasitol. Res.* **89**: 228-234.

Sudhakar, K.G. 2020. Molecular characterisation of Theileria orientalis and clinico-therapeutic studies of the infection in dairy cattle. Phd thesis, Kerala Veterinary and Animal Sciences University, Pookode, pp, 93.

Vinodkumar, K., Shyma, V., Justin, D.K., Ashok, S., Anu, J.P., Mini, K., Muhammedkutty, V., Sasidharan, S. and Chullipparambil, S. 2016. Fatal *Theileria orientalis* N2 genotype infection among Asian water buffaloes (*Bubalus bubalis*) in a commercial dairy farm in Kerala, India. *Parasitol.* **143***:* 69-74.

Von Schubert, C., Xue, G., Schmuckli-Maurer, J., Woods, K.L., Nigg, E.A. and Dobbelaere, D.A. 2010. The transforming parasite Theileria co-opts host cell mitotic and central spindles to persist in continuously dividing cells. *PLoS Biol.* **8**: 1000499.

Yagi, Y., Ito, N. and Kunugiyama, I. 1991. Decrease in erythrocyte survival in *Theileria sergenti*-infected calves determined by non-radioactive chromium labelling method. *J. Vet. Med. Sci.* **53**: 391-394.

Yagi, Y., Thongnoon, P., Shiono, H. and Chikayama, Y. 2002. Increase in oxidized proteins in *Theileria sergenti*-infected erythrocyte membrane. *J. Vet. Med. Sci*. **64**: 623-625. ■