



## SHORT COMMUNICATION

### Relationship between Packed Cell Volume Levels and Serum Nitric Oxide Concentrations in Cattle with Tropical Theileriosis

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#### ARTICLE HISTORY (14-317)

Received: June 19, 2014

Revised: August 18, 2014

Accepted: April 21, 2015

#### Key words:

Anaemia

Cattle

Nitric oxide

Packed cell volume

Theileriosis

#### ABSTRACT

This study was performed to determine changes in serum NO (nitric oxide) levels in cattle with theileriosis in accordance with PCV levels. For this purpose, 42 cattle with theileriosis and 10 healthy cattle have been used as material. Infected animals have been divided into 3 equal groups. 1<sup>st</sup> group had <12%, 2<sup>nd</sup> group had 13–24% and 3<sup>rd</sup> group had <25% PCV level. Blood samples have been used to determine NO concentrations. Serum NO concentrations prior to treatment increased in all infected groups compared to control group however its levels were significant only in the third group. NO concentrations decreased to almost normal values after treatment especially in 2<sup>nd</sup> and 3<sup>rd</sup> group, however this decrease was not significant in the first group. As a result, NO levels have been found increased in cattle with natural theileriosis. Therefore, its concluded that NO level and PCV levels in theileriosis should be studied with more detail in the future to enlighten the role of NO release and strategies for controlling its' release should be made especially in severe cases.

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**To Cite This Article:** Özkan C, N Altuğ, Y Akgül, İ Keleş, A Kaya, N Yüksek, ZT Ağaoğlu, M Özbek and F Yur, 2015. Relationship between packed cell volume levels and serum nitric oxide concentrations in cattle with tropical theileriosis. Pak Vet J, 35(3): 385-387.

#### INTRODUCTION

Theileriosis, is a protozoan disease transmitted by *Hyalomma* ticks which causes significant economical loss (Khan *et al.*, 2011). In theileriosis there are important changes observed at hematological and biochemical parameters causing various clinical findings. In recent years; Nitric oxide (NO) release in theileriosis, babesiosis and anaplasmosis has been reported (Jacobson *et al.*, 2002).

Nitric oxide is a free radical released from several cells and mediated by both physiological and pathological phenomenon (Ozkan, 2009). Since NO has short half-life, its' concentration is determined by measuring its' metabolites such as nitrite and nitrate (Mert *et al.*, 2009). NO is released at the beginning of diseases which kills intracellular parasites and this activity is very important in the control of infections. At the last stage of diseases, severe NO release causes important pathological disorders such as inflammation,

hypotension and immunosuppression in the host leading to more severe infection course (Ozkan, 2009). According to studies; cellular defense mechanisms are activated in theileriosis and NO is produced at high concentrations by being released from macrophages and monocytes which leads to protection against pathogens (Mert *et al.*, 2009).

Information given above led us to investigate NO concentration in cattle with natural theileriosis because studies concerning this subject were mainly in vitro and experimental (Ayerdem *et al.*, 2006). Furthermore, NO concentrations in various studies were different (Ergonul and Askar, 2009). In addition, there were no studies about NO concentrations in natural theileriosis cases which took PCV level into consideration. Thus, in this study comparison between NO concentrations and PCV levels has been aimed. Differences between NO concentrations obtained before and after treatment have been also discussed.

## MATERIALS AND METHODS

This study comprised of 10 healthy (control group) and 42 infected cattle brought to the University of Yuzuncu Yil, Animal Hospital, Faculty of Veterinary Medicine having natural theileriosis diagnosed after clinical and blood examination. The cattle had different ages, breeds and genders. Blood samples were taken from all animals before and after treatment and blood smears were stained with Giemsa. Cattle with clinical signs of theileriosis and presence of piroplasms under microscope were considered positive.

Blood samples were also subjected to determination of PCV using veterinary hematology device (QBC vetautoreader®-Idexx). In the light of literature information (Altug *et al.*, 2008); cattle with theileriosis were divided into 3 groups and each group had 14 animal according to their PCV level. Cattle having <12% PCV level formed 1<sup>st</sup> group, those having 13-24% PCV level formed 2<sup>nd</sup> group and cattle having >25% PCV level formed 3<sup>rd</sup> group. Blood samples were centrifuged at 3000 rpm (Rotofix 32®-Hettich) to separate serum which was then stored at -20°C until analysis.

For treatment of theileriosis; a single dose (2.5 mg/kg body weight) of buparvaquone (Butalex®-Ceva-DIF/Turkey) and vitamin B combination (Berovit B<sub>12</sub>®-Ceva-DIF/Turkey) were used for 5 days intramuscularly (1 ml/20 kg BW). Serum NO level was determined by Griess Reagent method, a commercial colorimetric method test kit (nitrate/nitrite colorimetric assay kit, Cayman Chemical Company, Catalog no: 780001), according to the kit procedure using ELISA device (ELISA reader®-DAS).

For statistical comparison between control and infected group (before and after treatment) values; independent-t test was used. Paired t-test has been used to compare differences within group before and after treatment. For statistical analysis SPSS 20.0 has been used. Differences between groups were determined by using Duncan test. P<0.05 values were considered statistically significant.

## RESULTS

Serum PCV and NO levels of control and infected groups has been given in Table 1 as mean and standard deviation. PCV level was decreased in cattle with theileriosis before treatment as compared to control group. Before and after treatment, PCV levels were significantly lower in 1<sup>st</sup> and 2<sup>nd</sup> group as compared to control group (P<0.05). PCV values after treatment were higher than the values measured before treatment, however the values were not statistically significant. In the comparison between the values before and after treatment; the increase was determined statistically significant only in the 1<sup>st</sup> group.

NO level was high in cattle with theileriosis before treatment compared to control group. However only in the 3<sup>rd</sup> group, these differences were significant. Furthermore, the values increased in diseased animals and fell to control values after treatment. When before and after treatment values were compared, a significant decrease in NO levels in 2<sup>nd</sup> and 3<sup>rd</sup> group animals were observed. In the comparison between before and after

treatment; in 2<sup>nd</sup> and 3<sup>rd</sup> groups, there were statistically significant decrease in NO levels.

## DISCUSSION

Relationship between theileriosis and NO has been evaluated in experimental and in vivo studies. However there are only limited studies concerning serum NO level and blood parasite infections (Mert *et al.*, 2009). El-Deeb and Younis (2009) studied theileriosis and NO levels in buffalo, but they did not evaluate NO levels concerning PCV levels. Therefore, the present study is the first report comparing PCV and NO levels in natural theileriosis. For this reason, the discussion will be focused on the relationship of PCV levels and changes in serum NO levels in cattle with theileriosis.

In several studies, various changes in hematological and biochemical parameters have been reported in cattle with theileriosis. The main reason for changes in theileriosis is progressive anemia and related disorders (Temiz *et al.*, 2014). As shown in Table 1; compared to control group, PCV level was low in cattle with theileriosis in the present study. PCV levels in this study; control group, group 1, group 2 and group 3 were detected 36.68, 10.10, 19.19 and 28.80%, respectively. Therefore, in all infected groups, due to the disease decreased PCV levels were determined.

In recent years, researchers reported that NO level increases in various parasitic diseases (Ergonul and Kontas Askar, 2009). NO release is favorable at the beginning of a disease, however; particularly at the late stage of a disease, NO release causes important pathological disturbances and worsen the severity of disease (Ozkan, 2009). Macrophages play an important role in the elimination and controlling of intracellular parasitic protozoa. This duty is accomplished by the release of various cytokines from macrophages (Mert *et al.*, 2009). During an acute infection, macrophages activate and these activated macrophages kill parasites by either phagocytosis or by production of some toxic mediators such as NO, peroxynitrite and superoxide (Ergonul and Kontas Askar, 2009).

Immunity to theileriosis is mainly cell mediated. NO is produced by a number of different cell types in response to cytokine stimulation. NO plays role in immunologically mediated protection against protozoan and helminthic parasites. Immunological response of the host is directed against all stages of theileriosis (Ahmed *et al.*, 2008).

In this study, NO concentrations increased in all infected groups. However, NO concentration was higher especially at the early stage of the disease which was observed in the 3<sup>rd</sup> group (5.24±1.18 µM). This shows that, in acute phase, NO release is higher. In natural theileriosis cases, cellular immune responses have been activated and high concentration of NO has been released from stimulated monocytes and macrophages (Mert *et al.*, 2009). Furthermore, in some studies (Jacobson *et al.*, 2002; Ayerdem *et al.*, 2006) NO concentrations were increasing especially in the last stages of disease.

NO levels of monocyte measured in cattle with theileriosis with acute course of a disease and cattle in agony were higher than healthy animals (Ayerdem *et al.*,

**Table 1:** Changes determined in serum nitric oxide levels in control and cattle with theileriosis group according to their PCV degree

Parameters	Control Group (n=10)	Animals with theileriosis			
			Group 1 (n=14)	Group 2 (n=14)	Group 3 (n=14)
Packed Cell Volume (%)	36.68±0.53 <sup>a</sup>	BT	10.10±0.74 <sup>c, #</sup>	19.19±1.60 <sup>bc</sup>	28.80±1.38 <sup>ab</sup>
		AT	16.87±0.56 <sup>bc, #</sup>	21.80±2.32 <sup>b</sup>	29.28±1.91 <sup>ab</sup>
Nitric oxide (µM)	1.34±0.44 <sup>b</sup>	BT	4.02±0.95 <sup>ab</sup>	3.95±0.85 <sup>ab, #</sup>	5.24±1.18 <sup>a, #</sup>
		AT	3.57±0.68 <sup>ab</sup>	2.29±0.92 <sup>b, #</sup>	1.42±1.01 <sup>b, #</sup>

BT: Before treatment, AT: After treatment; Mean±SE in the same row with different superscript differ significantly (P<0.05). #Difference between before and after treatment in the same group refer to significance (P<0.05).

2006). El-Deeb and Younis (2009) reported that NO concentrations decrease in buffalo with theileriosis but they did not make any evaluation concerning PCV levels. Furthermore, they have not been discussed reasons behind this reduction in that literature. None of any reports have been found related to comparing serum NO levels and PCV levels in cattle with theileriosis.

On the contrary, in the present study, serum NO concentration significantly increased in cattle with theileriosis. And its level decreased to almost control values after treatment. We think NO concentration might be increased due to infection as a defense mechanism. In this study, the resulting increase in NO levels, and reductions in NO levels after treatment, is in agreement with the literature information (Goff *et al.*, 2001). In this study, NO concentrations determined in cattle with theileriosis took PCV levels into consideration. In group 1 with low PCV level, NO concentration was not significantly high when compared to other groups (2<sup>nd</sup> and 3<sup>rd</sup>) and that was not in agreement with the findings of Ayerdem *et al* (2006). This situation might be resulted due to usage of different specimens for NO analysis. Because in the present study while serum samples have been used; in the other study (Ayerdem *et al.*, 2006) monocytes have been used.

When the results of the present study were evaluated according to researchers' findings (Mert *et al.*, 2009); determining high concentration of NO release in 3<sup>rd</sup> group might be the reflection of an acute cellular immunity at early stage of the disease. Furthermore, NO concentration decreased significantly after treatment in 2<sup>nd</sup> and 3<sup>rd</sup> groups, but not in 1<sup>st</sup> group which had very low PCV level. In addition, NO concentration in 1<sup>st</sup> group was still high after treatment and this situation supports the argument that release of NO helps the elimination of diseases especially at the beginning. However there are reports that NO may cause harmful effects at the late stage of diseases.

**Conclusion:** As a result; so far, NO levels in natural theileriosis infection have not been reported. This is the first report comparing PCV levels and NO concentration in natural theileriosis. NO release is useful in combating a disease at the early stage but might be harmful at the late stage of disease. Detailed studies concerning PCV level and NO release should be performed in the future. The

usefulness of the inhibition of NO release at late stage of a disease should also be investigated.

**Acknowledgement:** This study was supported by the Scientific Research Project Directory of the University of Yuzuncu Yil, Van, Turkey (Project No. 2007-VF-B04).

**Author's contribution:** CÖ, NA, YA designed the study and completed most of the works. CÖ, NA, AK, NY, ZTA, FY analyzed the test results and collected materials. CÖ, İK, MÖ did the writing of the manuscript. All authors read and approved the final manuscript.

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