

VETERINARSKI ARHIV 80 (2), 205-214, 2010

## Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with *Theileria annulata*

Saeed Nazifi<sup>1\*</sup>, Seyyed M. Razavi<sup>2</sup>, Mahdiah Reiszadeh<sup>2</sup>,  
Zahra Esmailnezhad<sup>2</sup>, and Maryam Ansari-Lari<sup>3</sup>

<sup>1</sup>Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

<sup>2</sup>Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

<sup>3</sup>Department of Food Hygiene, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

---

**NAZIFI, S., S. M. RAZAVI, M. REISZADEH, Z. ESMAILNEZHAD, M. ANSARI-LARI: Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with *Theileria annulata*. Vet. arhiv 80, 205-214, 2010.**

### ABSTRACT

This study was conducted to assess the pattern of changes and the relative value of acute phase proteins (APP) including haptoglobin (Hp), serum amyloid A (SAA), ceruloplasmin and fibrinogen in Iranian indigenous cattle infected with *Theileria annulata*. The diseased group comprised 24 Iranian indigenous dairy cattle, 2-3 years old, naturally infected with *Theileria annulata*. The infected animals were divided into 4 subgroups with different parasitemia rates (<1% and 1-3%). As a control group, 10 uninfected cattle were also sampled. Blood samples were collected and all measurements were made using validated methods. There were significant differences in red blood cells (RBCs), packed cell volume (PCV), hemoglobin (Hb) and concentrations of Hp, SAA, ceruloplasmin and fibrinogen between healthy cattle and those infected with *T. annulata* with different parasitemia rates ( $P < 0.05$ ). As the parasitemia rate increased in infected cattle, a significant decrease was observed in RBCs, PCV and Hb. In contrast, with the increase in the parasitemia rate, a significant increase in Hp, SAA, ceruloplasmin and fibrinogen was evident. The optimal cut-off point was set by the receiver operating characteristics (ROC) method to  $>5.68 \mu\text{g/mL}$  for SAA,  $>0.09 \text{ g/L}$  for Hp,  $>0.049 \text{ g/L}$  for ceruloplasmin and  $>1.90 \text{ g/L}$  for fibrinogen, with corresponding 71.50% sensitivity and 100% specificity for SAA, 83.30% sensitivity and 70% specificity for Hp, 50% sensitivity and 90% specificity for ceruloplasmin and 71.30% sensitivity and 80% specificity for fibrinogen. In conclusion, measuring SAA with the highest sensitivity, specificity and AUC compared to other APPs, can be a suitable indicator of inflammatory reactions in indigenous cattle infected with *Theileria annulata*.

**Key words:** haptoglobin, serum amyloid A, ceruloplasmin, fibrinogen, Iranian indigenous dairy cattle, *Theileria annulata*

---

\*Corresponding author:

Prof. Dr. Saeed Nazifi, Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, P.O. Box 1731, Shiraz 71345, Iran, Phone: +98 711 2286 940; Fax: +98 711 2286 950; E-mail: nazifi@shirazu.ac.ir

## Introduction

Tick-borne diseases are the most prevalent and numerous and exert their greatest impact in the tropical and subtropical regions (BRAM, 1983; MINJAUW and McLEOD, 2003). Theileriosis is one of the most important of these diseases. Tropical theileriosis is one of the most prevalent and economically important fatal diseases of cattle in Iran (HASHEMI-FESHARKI, 1988). It is a progressive lymphoproliferative disease of cattle caused by the protozoan parasite, *Theileria annulata* (OMER et al., 2003a and 2003b; TAYLOR et al., 1992). The parasite acts as a serious constraint to cattle production in endemic areas, causing lethal infections in exotic cattle and considerable mortality in indigenous and crossbred stock (FORSYTH et al., 1997).

The acute phase response is a non-specific reaction by an individual to different types of tissue damage (GRUYS et al., 1994). The acute phase proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress. The APPs consist of negative and positive proteins that show a decrease and an increase in levels, respectively, in response to challenge (ECKERSALL, 2004; MURATA et al., 2004; GRUYS et al., 2005). The wide nature of acute phase protein response can be seen as a disadvantage in that the APP assay is not specific for one disease, but it is combined with other long-established diagnostic tests in the clinicians' repertoire (ECKERSALL, 2004). On the other hand, it is suggested that various infections and inflammatory processes may be associated with different APP pattern details, which may lead to APP parameters being used for diagnostic purposes (GRUYS et al., 2005). GLASS et al. (2003) reported that *T. annulata* causes severe pathology in susceptible cattle by inducing high levels of pro-inflammatory cytokines. In another study, GLASS et al. (2005) reported that *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *T. annulata* and produce markedly different levels of APPs. GLASS and JENSEN (2007) reported that *Bos indicus*, Sahiwal, which originates in a *T. annulata* endemic area, is more resistant to the parasite. One feature of the disease is the production of APPs, indicating that the parasite induces high systemic levels of pro-inflammatory cytokines. In theileriosis, leukocyte composition is not a suitable indicator of inflammatory progress since variations in leukocytes in different stages of the disease range between normal numbers of leukocyte to leukocytosis or leukopenia. Acute phase proteins (APP) have been identified as the biomarkers of choice for diagnostic and prognostic purposes in veterinary medicine, therefore evaluation of these proteins is considered to be a more helpful indicator of inflammatory progress.

The present study was conducted to assess the pattern of changes and the relative value of APP, including Hp, SAA, ceruloplasmin and fibrinogen and to find a possible relationship between parasitemia rates and APP changes in indigenous cattle infected with *Theileria annulata*.

### Materials and methods

This study was carried out in the south west region of Iran (Fars province) where theileriosis due to *T. annulata* is prevalent. Blood samples were collected from 24 indigenous dairy cattle, 2-3 years old, which were naturally infected with *Theileria annulata*. Ten healthy indigenous cattle were selected as the control group. The theileria infected group was divided into 2 subgroups according to their parasitemia rates (<1% and 1-3%). Theileria infection was diagnosed based on hematological, biochemical and microbiological tests, clinical signs and epidemiological evidences. Other causes of anemia were excluded using above-mentioned tests. Infected animals had not been treated for disease prior to sampling and were sampled only once in the course of their disease.

Blood samples were taken from jugular vein into vacutainers containing EDTA for measuring hematological parameters and without EDTA for biochemical analysis. The sera were separated by centrifugation at 750g for 15 min and stored at -20 °C until used. Hematological parameters including RBC count, WBC differential count, PCV value, hemoglobin concentration, MCV, MCH and MCHC were measured by routine procedures (JAIN 1986). Thin blood smears were prepared, fixed with absolute methanol (5min), stained with 10% Giemsa solution (30 min) and examined under oil immersion lens ( $\times 1000$ ) to observe intraerythrocytic forms of *Theileria annulata*. After examining more than 50 microscopic fields of blood films at a magnification of  $\times 1000$ , the parasitemia rate was quantified and expressed as the percentage of infected erythrocytes. Haptoglobin (Hp) was measured using a solid phase ELISA (Tridelta Development Plc, Co. Wicklow, Ireland) and SAA by a solid phase sandwich ELISA (Tridelta Development Plc, Co. Wicklow, Ireland). The analytical sensitivities of these tests in serum have been determined as 0.3  $\mu\text{g/mL}$  for SAA and 0.0156 mg/mL for Hp by the manufacturer. Ceruloplasmin was measured using the SUNDERMAN and NOMOTO (1970) method and fibrinogen by precipitation-refractory method as described by THRALL (2004).

The Student's *t*-test was used for comparison of factors between the 2 groups (control and diseased cases). Analysis of variance (ANOVA) and Tukey tests were used for comparison of factors in all subgroups with different parasitemia rates, and Pearson's correlation coefficient was used for the relationship between the parameters at different parasitemia rates. Selection of cut-off points for Hp, SAA, ceruloplasmin and fibrinogen was undertaken using the receiver operating characteristic curve (ROC) analysis. ROC analysis plots a curve of sensitivity versus specificity for all possible threshold values of the parameter to be evaluated. The area under the curve (AUC) indicates the diagnostic accuracy of the examined parameter (GARDNER and GREINER, 2006). Likelihood ratios (LR) for Hp, SAA, ceruloplasmin and fibrinogen were also estimated. LR provides an indication of how the post-test probability changes as a function of magnitude of the test

results (GARDNER and GREINER, 2006). For a better evaluation of the diagnostic value of study parameters, ROC analysis was performed twice, including and excluding the control group. All values were expressed as mean and standard error (SE), and  $P < 0.05$  was considered as statistically significant.

### Results

The mean  $\pm$  SEM of hematological parameters and APPs concentrations in indigenous healthy cattle and those infected with *Theileria annulata* with different parasitemia rates are presented in Table 1.

There were significant differences in RBC, PCV, Hb and concentrations of Hp, SAA, ceruloplasmin and fibrinogen between healthy cattle and those infected with *T. annulata* with different parasitemia rates ( $P < 0.05$ ). As the parasitemia rate increased in indigenous infected cattle, a significant decrease was observed in white blood cells (WBCs) ( $r = -0.345$ ,  $P < 0.01$ ), percentages of lymphocytes ( $r = -0.424$ ,  $P < 0.01$ ), RBCs ( $r = -0.426$ ,  $P < 0.01$ ), PCV ( $r = -0.893$ ,  $P < 0.01$ ) and Hb ( $r = -0.788$ ,  $P < 0.01$ ). In contrast, with the increase in parasitemia rates, a significant increase in neutrophils ( $r = 0.307$ ,  $P < 0.01$ ), Hp ( $r = 0.779$ ,  $P < 0.01$ ), SAA ( $r = 0.730$ ,  $P < 0.01$ ), ceruloplasmin ( $r = 0.772$ ,  $P < 0.01$ ) and fibrinogen ( $r = 0.691$ ,  $P < 0.01$ ) was evident. No significant increase in the percentage of monocyte was observed as the parasitemia rates increased.

The cut-off point, sensitivity, specificity, positive and negative likelihood ratio and area under the ROC curve (AUC) are shown in Table 2. The optimal cut-off point was set by the ROC method to  $> 5.68 \mu\text{g/mL}$  for SAA,  $> 0.09 \text{ g/L}$  for Hp,  $> 0.049 \text{ g/L}$  for ceruloplasmin and  $> 1.90 \text{ g/L}$  for fibrinogen with corresponding 71.50% sensitivity and 100% specificity for SAA, 83.30% sensitivity and 70% specificity for Hp, 50% sensitivity and 90% specificity for ceruloplasmin and 71.30% sensitivity and 80% specificity for fibrinogen. The sensitivity, specificity and the area under the ROC curve (AUC) of SAA were more than that of Hp, ceruloplasmin and fibrinogen (Table 2).

Table 2. ROC analysis of Hp, SAA, ceruloplasmin and fibrinogen in indigenous cattle naturally

Table 1. Mean  $\pm$  SE of hematological parameters and APPs concentrations in indigenous healthy cattle and those infected with *Theileria annulata* with different parasitemia rates

Group	Parasitemia rate	N <sup>o</sup>	WBC		Neutrophils		Lymphocytes		Monocytes		Eosinophils		Band	
			10 <sup>9</sup> /L	%	10 <sup>9</sup> /L	%	10 <sup>9</sup> /L	%	10 <sup>9</sup> /L	%	10 <sup>9</sup> /L	%	10 <sup>9</sup> /L	%
Uninfected	0	10	6.46	32.70	2.11	63.00	4.06	3.30	0.21	1.00	0.06	0.00	0.00	0.00
			$\pm 0.37$	$\pm 2.90$	$\pm 0.12$	$\pm 3.30$	$\pm 0.23$	$\pm 0.80$	$\pm 0.01$	$\pm 0.29$	$\pm 0.00$	$\pm 0.00$	$\pm 0.00$	$\pm 0.00$
Infected	<1	13	4.59 <sup>a</sup>	36.92	1.69	59.77	2.74	2.69	0.12	0.54	0.02	0.007	0.0003	0.0003
			$\pm 0.61$	$\pm 1.92$	$\pm 0.08$	$\pm 2.18$	$\pm 0.10$	$\pm 0.49$	$\pm 0.02$	$\pm 0.27$	$\pm 0.01$	$\pm 0.007$	$\pm 0.0003$	$\pm 0.0003$
Infected	1-3	11	4.01 <sup>a</sup>	53.64 <sup>a</sup>	2.05 <sup>a</sup>	43.27 <sup>b</sup>	1.73 <sup>a</sup>	2.73	0.10	0.27	0.01	0.009	0.0003	0.0003
			$\pm 0.28$	$\pm 5.32$	$\pm 0.21$	$\pm 5.37$	$\pm 0.21$	$\pm 0.19$	$\pm 0.007$	$\pm 0.19$	$\pm 0.007$	$\pm 0.009$	$\pm 0.0003$	$\pm 0.0003$

  

Group	Parasitemia rate	N <sup>o</sup>	RBC	PCV	Hb	MCV	MCH	MCHC	Hp	SAA	Ceruloplas-	Fib
Uninfected	0	10	6.64	0.29	99.10	43.84	14.83	338.5	0.08	4.50	0.06	1.69
			$\pm 0.23$	$\pm 0.01$	$\pm 5.40$	$\pm 0.97$	$\pm 0.31$	$\pm 2.0$	$\pm 0.01$	$\pm 0.18$	$\pm 0.00$	$\pm 0.10$
Infected	<1	13	5.78 <sup>a</sup>	0.25 <sup>a</sup>	86.50 <sup>a</sup>	44.17	14.96	338.7	0.09	4.98	0.03 <sup>a</sup>	1.88
			$\pm 0.008$	$\pm 0.02$	$\pm 1.10$	$\pm 0.24$	$\pm 0.009$	$\pm 13.2$	$\pm 0.00$	$\pm 0.26$	$\pm 0.00$	$\pm 0.11$
Infected	1-3	11	5.48 <sup>b</sup>	0.22 <sup>b</sup>	77.50 <sup>b</sup>	40.55 <sup>a</sup>	14.17 <sup>a</sup>	349.5 <sup>a</sup>	0.21 <sup>a</sup>	12.09 <sup>a</sup>	0.09 <sup>b</sup>	2.57 <sup>a</sup>
			$\pm 0.15$	$\pm 0.02$	$\pm 0.90$	$\pm 0.44$	$\pm 0.19$	$\pm 2.00$	$\pm 0.02$	$\pm 1.51$	$\pm 0.00$	$\pm 0.10$

Comparisons with P>0.05 are not represented by a letter. <sup>a</sup> = significant difference compared to control animals (P<0.05). <sup>b</sup> = significant difference compared to less severely infected animals (P<0.05)

infected by *Theileria annulata* (n = 24)

Parameter	Cut-off-point	Sensitivity (%)	Specificity (%)	AUC	LR+	LR-	P value
Hp (g/L)	>0.09	83.30	70.00	0.796	2.78	0.24	0.0001
SAA (µg/mL)	>5.68	71.50	100.00	0.822		0.37	0.0001
Ceruloplasmin (g/L)	>0.049	50.00	90.00	0.570	5.00	0.56	0.49
Fibrinogen (g/L)	>1.90	71.30	80.00	0.780	4.17	0.21	0.0001

+ LR= Positive likelihood ratio; -LR = Negative likelihood ratio

### Discussion

In the present study, the numbers of RBC, PCV and Hb in Iranian indigenous cattle with theileriosis were significantly lower in comparison with healthy ones ( $P < 0.05$ ). Also, as the severity of parasitemia progressed, a significant decrease in RBC, PCV and Hb was observed ( $P < 0.05$ ). Therefore, the severity of the anemia increased as the parasitemia progressed. These findings were similar to those of DHAR and GAUTAM (1979), SHARMA (1979), MEHTA et al. (1988), RAYULA and HAFEEZ (1995), SANDHU et al. (1998) and SINGH et al. (2001). The decline in RBC, PCV and Hb may be attributed to the destruction of erythrocytes by macrophages in the lymph nodes, spleen and other organs of the monocyte-macrophage system (SINGH et al., 2001). SANDHU et al. (1998) evaluated hematological and biochemical factors in experimental *Theileria annulata* infection in crossbred calves. They reported a significant progressive decrease in Hb concentration, PCV and RBC, whereas the total leukocyte count showed initial non-significant leukocytosis followed by significant leukopenia. DHAR and GAUTAM (1979), SHARMA (1979), MEHTA et al. (1988) and RAYULA and HAFEEZ (1995) reported a progressive decrease in Hb, PCV and RBC in acute *Theileria annulata* infection. A progressive decrease in the Hb and PCV, along with a marked reticulocytosis was also reported by SINGH et al. (2001). No significant difference was observed in MCV and MCHC between infected and healthy indigenous cattle that indicated normocytic-normochromic anemia in infected cattle. STOCKHAM et al. (2000) reported macrocytic-normochromic anemia in cattle infected with *Theileria buffeli*.

Inflammation or tissue injury causes the release of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF $\alpha$ ), which alters the blood concentration of a variety of proteins that are produced primarily in the liver. Overall

increases in these proteins are recognized by finding elevations in the  $\alpha$ -and/or  $\beta$ -regions by serum protein electrophoresis. The concentration of these proteins is generally low to non-detectable in healthy animals, and elevations are used to diagnose and monitor inflammatory diseases. The specific type of APPs and the time course for alterations in these proteins varies between different species on the basis of the initiating disorder or underlying inflammatory process (FELDMAN et al., 2000). Hemorrhage, lymphocyte infiltration and necrosis in abomasum, liver, kidney, intestine, lung and bone marrow indicates a wide spread inflammatory reaction in bovine theileriosis. These lesions induce cytokines such as IL-1, IL-6 and TNF $\alpha$  under the effect of which, APPs are synthesized in the liver (JUBB et al., 1991; RADOSTITS et al., 2007).

There was a significant difference in Hp, SAA, ceruloplasmin and fibrinogen between healthy and diseased Iranian indigenous cattle ( $P < 0.05$ ). As parasitemia progressed, a significant elevation in Hp, SAA, ceruloplasmin and fibrinogen was evident. In comparison to SAA, ceruloplasmin and fibrinogen had lower sensitivity and specificity in cattle infected with *Theileria annulata*. Ceruloplasmin concentrations increase in some, but not all, spontaneous inflammatory diseases. Ceruloplasmin transports copper and plays a role in iron metabolism. Although less commonly measured than Hp, ceruloplasmin has been identified as an acute phase protein in many species (FELDMAN et al., 2000). Fibrinogen levels increase with a variety of spontaneous inflammatory conditions in many species. It is commonly measured as part of a routine complete blood count in cows because it may be a better indicator of inflammation in some situations than alterations in leukocyte counts (FELDMAN et al., 2000; LATIMER et al., 2003). The sensitivity and specificity of SAA were more than Hp, ceruloplasmin and fibrinogen for inflammatory reactions of indigenous cattle infected with *Theileria annulata*. APPs increase when a disease developed and decrease in recovery stage. SAA with the highest sensitivity and specificity in comparison with other APPs is a more suitable diagnostic indicator of inflammatory process in bovine theileriosis. GLASS et al. (2003) reported that *Theileria annulata* causes severe pathology in susceptible cattle by inducing high levels of pro-inflammatory cytokines. Following experimental infection, SAA appeared first, followed by a rise in  $\alpha_1$ -acid glycoprotein in all cattle, whereas Hp, which is a major APP in cattle, only appeared in some of the animals and generally at a low level. SAA, Hp and  $\alpha_1$ -acid glycoprotein only elevated around or after the appearance of schizonts and after the first rise in temperature. Increased  $\alpha_1$ -acid glycoprotein levels coincided with the appearance of piroplasms. The production of SAA and  $\alpha_1$ -acid glycoprotein correlated strongly with each other, and also with some clinical features of disease severity, including the time of fever, development of leukopenia, parasitemia rate and mortality. GLASS et al. (2005) reported that *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *T. annulata* and produce markedly different levels of APPs. Following experimental infection with *T. annulata* sporozoites, a group of Sahiwal calves all survived without

treatment, with significantly lower maximum temperatures and a lower parasitemia rate than a group of Holstein calves, which all showed severe clinical manifestation. Although the Sahiwals were as anemic as the Holsteins, other pathologic lesions, including lymphadenopathy and the level of APPs,  $\alpha_1$  acid glycoprotein and Hp, were significantly lower in the Sahiwals. Additionally, the Sahiwals had significantly lower resting levels of  $\alpha_1$  acid glycoprotein than the Holsteins.

In conclusion, measuring SAA with the highest sensitivity, specificity and AUC compared to the other APPs, can be a suitable indicator of inflammatory reactions in indigenous cattle infected with *Theileria annulata*.

### References

- BRAM, R. A. (1983): Tick-borne livestock diseases and their vectors: the global problem. In: Ticks and Tick-borne Diseases, FAO Animal Production and Health Paper. (Food and Agricultural Organization, Rome). 36, 7-11.
- DHAR, S., O. P. GAUTAM (1979): Observations on anemia in experimentally induced *Theileria annulata* infection of calves. Indian. J. Anim. Sci. 49, 511-516.
- ECKERSALL, P. D. (2004): The time is right for acute phase protein assays. Vet. J. 168, 3-5.
- FELDMAN, B. V., J. G. ZINKL, N. C. JAIN (2000): Schalm's Veterinary Hematology, 5<sup>th</sup> ed. Williams and Wilkins. Philadelphia, pp. 1152-1159.
- FORSYTH, L. M. G., L. A. JACKSON, G. WILKIE, A. SANDERSON, C. G. D. BROWN, P. M. PRESTON (1997): Bovine cells infected *in vivo* with *Theileria annulata* express CD11b, the C<sub>3</sub>bi complement receptor. Vet. Res. Comm. 21, 249-263.
- GARDNER, I. A., M. GREINER (2006): Receiver-operating characteristic curves and likelihood ratios: improvements over traditional methods for evaluation and application of veterinary clinical pathology tests. Vet. Clin. Pathol. 35, 8-17.
- GLASS, E. J., S. C. GRAIGMILE, A. SPRINGBETT, P. M. PRESTON, E. KIRVAR, G. M. WILKIE, P. K. ECKERSALL, F. R. HALL, C. G. BROWN (2003): The protozoan parasite *Theileria annulata* induces a distinct acute phase protein response in cattle that is associated with pathology. Int. J. Parasitol. 33, 1409-1418.
- GLASS, E. J., P. M. PRESTON, A. SPRINGBETT, S. CRAIGMILE, E. KIRVAR, G. WILKIE, C. G. BROWN (2005): *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. Int. J. Parasitol. 35, 337-347.
- GLASS, E. J., K. JENSEN (2007): Resistance and susceptibility to a protozoan parasite of cattle- Gene expression differences in macrophages from different breeds of cattle. Vet. Immunol. Immunopathol. 120, 20-30.
- GRUYS, E., M. J. OBWOLO, M. J. M. TOUSSAINT (1994): Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Vet. Bull. 64, 1009-1018.



- GRUYS, E., M. J. M. TOUSSAINT, T. A. NIEWOLD, S. J. KOOPMANS (2005): Acute phase reaction and acute phase proteins. *J. Zhejiang. Univ. Sci. B.* 6, 1045-1056.
- HASHEMI-FESHARKI, R. (1988): Control of *Theileria annulata* in Iran. *Parasitol. Today.* 4, 36-40.
- JAIN, N. C. (1986): *Schalm's Veterinary Hematology*. 1<sup>st</sup> ed. Lea and Febiger. Philadelphia, pp. 20-80.
- JUBB, K. V. F., P. C. KENEDY, N. PALMER (1991): *Pathology of Domestic Animals*. 4<sup>th</sup> ed. Vol 3, Academic Press. California, pp. 251-255.
- LATIMER K. S., E. A. MAHAFFEY, K. W. PRASSE (2003): *Veterinary Laboratory Medicine. Clinical Pathology*. 4<sup>th</sup> ed. Iowa State Press. Iowa, pp. 3-45, 260-270.
- MEHTA, H. K., R. S. SISODIA, R. S. MISRAULA (1988): Clinical and haematological observations in experimentally induced cases of bovine theileriosis. *Indian J. Anim. Sci.* 58, 584-587.
- MINJAUW, B., A. McLEOD (2003): Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Center for Tropical Veterinary Medicine, University of Edinburgh, UK.
- MURATA, H., N. SHIMADA, M. YOSHIOKA (2004): Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet. J.* 168, 28-40.
- OMER, O. H., K. H. EI-MALIK, M. MAGZOUB, O. M. MAHMOUD, E. M. HAROUN, A. HAWAS, H. M., OMAR (2003a): Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. *Vet. Res. Comm.* 27, 15-25.
- OMER, O. H., E. M. HAROUN, O. M. MAHMOUD, E. M. ABDEL-MAGIED, K. H. EI-MALIK, M. MAGZOUB (2003b): Parasitological and clinicopathological profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. *J. Vet. Med. B.* 50, 200-203.
- RADOSTITS, O. M., C. C. GAY, K. W. HINCHCLIFF, P. D. CONSTABLE (2007): *Veterinary Medicine*. 10<sup>th</sup> ed. W. B. Saunders Co, Philadelphia pp. 1529-1531.
- RAYULA, V., M. D. HAFEEZ (1995): Haematological values in cattle infected with *Theileria annulata*. *Indian. J. Anim. Sci.* 65, 1202-1203.
- SANDHU, G. S., A. S. GREWAL, A. SINGH, J. K. KONDAL, J. SINGH, R. S. BRAR (1998): Haematological and biochemical studies in experimental *Theileria annulata* infection in crossbred calves. *Vet. Res. Comm.* 22, 347-354.
- SHARMA, N. N. (1979): Hematological observations in bovine theileriosis, anaplasmosis and in mixed infections. *Indian. J. Parasitol.* 3, 153-156.
- SINGH, A., J. SINGH, A. S. GREWAL, R. S. BRAR (2001): Studies on some blood parameters of crossbred calves with experimental *Theileria annulata* infections. *Vet. Res. Comm.* 25, 289-300.
- STOCKHAM, S. L., A. M. KJEMTRUP, P. A. CONRAD, D. A. SCHMRIDT, M. A. SCOTT, T. W. ROBINSON, J. W. TYLER, G. C. JOHNSON, C. A. CARSON, P. CYDDUGEEM (2000):

S. Nazifi et al.: Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with *Theileria annulata*

Theileriosis in a Missouri beef herd caused by *Theileria buffeli*: case report, herd investigation ultrastructure, phylogenetic analysis and experimental transmission. *Vet. Pathol.* 37, 11-21.

SUNDERMAN, F. W. Jr., S. NOMOTO (1970): Measurement of human serum ceruloplasmin by its *p*-phenylenediamine oxidase activity. *Clin. Chem.* 160, 903-907.

TAYLOR, S. M., A. G. HUNTER, A. H. ANDREW, R. W. BLOWERY, H. OYD, R. G. EDDY (1992): Tick and arthropod-borne diseases. In: *Bovine Medicine, Diseases and Husbandry of Cattle*, Blackwell Scientific Publications. Cambridge, pp. 722-757.

THRALL, M. A. (2004): *Veterinary Hematology and Clinical Chemistry*, Lippincott Williams and Wilkins. Philadelphia, pp. 401-415.

Received: 17 January 2009

Accepted: 29 January 2010

---

**NAZIFI, S., S. M. RAZAVI, M. REISZADEH, Z. ESMAILNEZHAD, M. ANSARI-LARI: Dijagnostičke vrijednosti proteina akutne faze u iranskoga domaćega goveda invadiranoga praživotinjom *Theileria annulata*. *Vet. arhiv* 80, 205-214, 2010.**

**SAŽETAK**

Istraživanje je provedeno s potrebom da se utvrdi dinamika promjena i relativne vrijednosti proteina akutne faze, uključujući haptoglobin (Hp), serumski amiloid A, ceruloplazmin i fibrinogen, u iranskoga domaćega goveda invadiranoga praživotinjom *Theileria annulata*. Skupina pokusnih životinja sadržavala je ukupno 24 iranska domaća mliječna goveda u dobi od dvije do tri godine invadirana praživotinjom *Theileria annulata*. Invadirane životinje bile su podijeljene u četiri podskupine s obzirom na različite razine parazitacije (<1% i 1-3%). U kontrolnoj skupini bilo je 10 neinvadiranih životinja. Izvađena krv bila je pretražena validiranim metodama. Ustanovljene su značajne razlike u broju eritrocita, ukupnoga staničnoga volumena, hemoglobina te koncentraciji haptoglobina, serumskog amiloida A, ceruloplazmina i fibrinogena između zdravih goveda i onih invadiranih s različitim razinama parazitacije ( $P < 0,05$ ). Jačanjem parazitacije u goveda dokazan je i značajan pad broja eritrocita, vrijednosti hematokrita i hemoglobina. Suprotno, jačanjem parazitacije zabilježen je značajan porast koncentracije haptoglobina, serumskog amiloida A, ceruloplazmina i fibrinogena. Optimalna granična vrijednost između pozitivnih i negativnih vrijednosti bila je određena pomoću krivulje ROC (engl. receiver operating characteristics) te je iznosila  $>5,68 \mu\text{g/mL}$  za serumski amiloid A,  $>0,09$  za haptoglobin,  $>0,049 \text{ g/L}$  za ceruloplazmin i  $>1,90 \text{ g/L}$  za fibrinogen. Za serumski je amiloid osjetljivost iznosila 71,50% dok je specifičnost bila 100%. Osjetljivost je za haptoglobin iznosila 83,30% dok je specifičnost iznosila 70%. Za ceruloplazmin je osjetljivost iznosila 50%, a specifičnost 90%. Za fibrinogen je osjetljivost iznosila 71,30% dok je specifičnost iznosila 80%. Zaključno se može reći da mjerenje vrijednosti serumskog amiloida A može biti prikladan pokazatelj upale uzrokovane praživotinjom *Theileria annulata* jer se u odnosu na ostale proteine akutne faze odlikuje najvišom razinom osjetljivosti, specifičnosti i AUC.

**Ključne riječi:** haptoglobin, serumski amiloid A, ceruloplazmin, fibrinogen, iransko domaće govedo, *Theileria annulata*

---