

Original research

Clinicopathological studies on camels (*Camelus dromedaries*) infected with Theileriosis in Aswan Governorate

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Received: 23/6/2022

Accepted: 4/7/2022

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Abstract:

Camel is an ancient species reared for different purposes since long time ago. In spite of cruel environmental conditions exposed to camels, it has the ability to sustain. Whilst, hemoprotozoans are diseases significantly upset camel's health induced economic loss. Theileria is a vector borne hemoparasites resulted in fatal financial losses. However, current consideration planned for investigation of hemato-biochemical and histopathological changes induced in camels during haemoparasitism. One hundred camels were under study from the slaughter houses in Aswan Province. During October 2019 to September 2020, blood samples were collected from jugular veins for complete hematological picture and biochemical assays. Moreover, samples from lymph nodes and liver were taken and fixed in 10 % neutral buffered formalin for further pathological examinations. From the survey results, our study investigated that about 15 % of camels were positive for Theileria infection. Generally, Theileria spp. was represented the highest incidence of infection in autumn (8%), and the lowest incidence of infection through different seasons was represented in spring only (1%) Theileria spp. Hematologically, Theileria was significantly decreased red blood cell (RBCs) count, hemoglobin (Hb.) concentration and packed cell volume (PCV) in comparison with non-infected camels. Biochemical results revealed significant elevation in serum aspartate aminotransferase (AST) and urea in Theileria infected camels when compared with non-infected camels. Histopathologically, Theileria infected camel's revealed necrosis of the lymphoid follicles and vacuolar degeneration in the hepatocytes. From the previous findings, it could be concluded that Theileriosis manifested with hemato-biochemical alterations, additionally histological deteriorations among slaughtered camels.

Keywords: Camel, Theileria, Hematologically, Biochemically, Histopathology.

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1- Introduction

In ancient time, camel is inhabitant arid and semi-arid countries. Noteworthy, camels are famous for hard work and have ability to tolerate the harsh environmental conditions (Farooq et al., 2012). However, during the last two decades, scientific investigations recorded more than 50% mortalities between camelids suffered from infectious diseases.

The genus *Theileria* are the vector-borne protozoan belongs to the Apicomplexa phylum that occupied *Babesia* species (Radostits et al., 2007). Theileriosis is considered as one of the most exhausting hemoparasites affecting ruminant induced fatal infections (El-Metenawy, 2000). Tropical theileriosis caused by genus *Theileria* of a wide ranged distribution extending from Africa to China (Mukhebi et al., 1992). But, there was scarce attention about Theileriosis disease in camels. *Theileria camelensis* and *T. dromedarii* are the most two frequent species among *Theileria* spp. have been recorded in camels in Egypt, Turkmenistan and Somalia. Mixed hemoparasites infections with different genus may occur (Ismael et al., 2014).

Theileriosis is of global significance and characterized by high economic impact attributed to severe clinical manifestations as death, besides anemia, icterus, and hemoglobinuria (Wagner et al., 2002). Furthermore, *Theileria* infected camels are manifested signs graded from gastrointestinal to systemic including fever, severe emaciation, and diarrhea, besides enlargement of the lymph nodes were also noticed (El-Fayoumy et al., 2005; Hamed et al., 2011).

Consequently, *Theileria* conducted deleterious effect on camel's health. Since, it induced hematobiochemical alterations like significant reduction in the total RBC count, Hb. concentration, hematocrit (HCT) and mean cell volume (MCV) between the affected camels. In addition, significant increases were detected in platelets. Biochemically, *Theileria* disturbs hepato-renal functions leading to high increment in Gamma glutamate (GGT), AST, ALT, total bilirubin, and blood urea nitrogen of the affected camels (Ismael et al., 2014).

2- Materials and Methods

A- Materials:

- **Survey camels:**

In this study, a total of 100 of dromedary camel herd within 3-7 years old from Daraw and Aswan slaughter houses, belonging to Aswan Governorate during period from October 2019 to September 2020. The survey camels appeared normal even infected ones.

B- Methods:

1- Blood sampling:

Under controlled conditions, blood samples dragged from jugular vein and divided into two parts. First sample collected in anticoagulated vacuum tube. While second one was taken in plain tubes; then centrifuged at 3000 rpm for 10 minutes. The resultant serum were collected in clean epindorff's tubes and stored at -20° C until biochemical assay.

- **Hematology:**

Stained blood smears performed for parasitological examination. Furthermore, complete blood picture was done using Automatic Blood Cell Counter (BC-2800 Vet Analyzers- China) (Feldman et al. 2000) in Animal Health Research Institute, Dokki, Giza.

- **Biochemical assay:**
- **Determination of liver functions tests:**

Activity of AST and alanine aminotransferase (ALT) was evaluated in serum using Biodiagnostic kits (Biodiagnostic Company, Giza, Egypt) according to Reitman and Frankel (1957).

Assessment of total protein performed via colorimetric method as described by Gornal et al. (1949). Likewise, colorimetric method of albumin was done in parallel with Doumas et al. (1971). While, globulin values were assessed through subtraction the albumin values from total protein values.

- **Determination of kidney function tests:**

Colorimetric method used for determination of urea as mentioned by Fawcett and Scott (1960). Creatinine level was tested colorimetrically method described by Bartles et al. (1972).

2- Histopathological examinations:

Lymph nodes and liver biopsies from the *Theileria* infected camels dissected and fixed in 10 % neutral-buffered formalin. The specimens were undergone tissues processing and paraffin embedding technique. Sections about 5 μ m were taken to be stained with the Harris haematoxylin and eosin stain (HE.) (Bacha and Bacha, 2000).

3 - Statistical analysis:

Statistical Package for Social Sciences Program using SPSS (version 17, SPSS Inc., Chicago, IL, USA) was used (Borenstein et al., 1997). Data are expressed as the Mean \pm SD. Comparisons among groups were tested using an analysis of variance (ANOVA). Differences were significant at $P < 0.05$.

3- Results and discussion:

Parasitic diseases particularly tick-borne hemoprotozoans are hazardously possess influences on animal health and productivity. Hemoprotozoan constitutes public health hazards correlated with socio-economic troubles mainly in Africa (Mohammed and Elshahawy, 2018). Among tick-borne protozoan, theileriosis - (Radostits et al., 2007), whereas it is clinically important attributed to occurrence of the high mortality among the infected animals during haemoparasitism (Mahran, 2004). Theileriosis is one of the most subversive blood parasites causing fatality in exotic cattle (El-Metenawy, 2000).

In our study, microscopical examination of the Giemsa stained blood smears revealed *Thieleria* spp. in (15%) out of the 100 asymptomatic camels examined throughout four different seasons (From October 2019 till September 2020). Generally, *Thieleria* spp. was represented the highest incidence of infection in autumn (8%) as associated with spreading of the ticks and insects, and the lowest incidence of infection through different seasons was represented in spring only (1%) *thieleria* spp. Parasitological findings, the blood film showed *Theileria* in cocci and signet ring form within the erythrocytes of 15 % the examined camels (**Fig. 1**). However, Giemsa-stained blood film displayed positive infection with *Thieleria* piroplasm in cocci form within erythrocyte (**Fig. 1a**), dacrocyte cell in *T. annulata* with signet-ring (**Fig. 1b**), and Schizont stages detected within the infected lymphocytes in form of mass of light-bluish bodies (**Fig. 1c**). *Theileria* produced many schizonts and piroplasms in RBCs of the infected camels which demonstrate the high pathogenicity and the 1st introduction of theileriosis in animals (Nassar,

1992). Theileria has various developmental stages of different shapes and forms inside this vector (Salimabadi et al., 2010; Hamed et al., 2011).

Haematologically, There mean values of RBCs count, Hb. Concentration, and PCV % revealed significant reduction ($P<0.05$) in in Theileria infected camels when compared with non-infected camels (**Table, 1**). Whereas the other haematological parameters including WBC, count, MCH, and MCHC exhibited non-significant changes in Theileria infected camels when compared with non-infected camels (**Table, 1**).

It was suggested that Theileria is capable of the removal of erythrocytes by phagocytosis rather than it induced lysis to erythrocytes cell membrane due to multiple invasion of parasite to the RBCs (Boulter and Hall, 2000). When Theileria parasitized erythrocytes, further destruction of erythrocytes occur (Mohandas and An, 2012). Theileria infection resulted in clinical, hematological and pathological alterations (Kawamura et al., 1987).

From a biochemical aspect, Theileria-infected camels showed significant increases in AST level ($P<0.05$) in comparison with non-infected camels (**Table, 2**). While activity of ALT, exhibited non-significant changes when compared with non-infected camels (**Table, 2**). Total protein, albumin and globulin levels displayed non-significant changes in Theileria infected camels when compared to non-infected animals (**Table, 3**). The mean values of the urea were highly elevated ($P<0.05$) in Theileria infected camels when compared to non-infected camels (**Table, 4**). However, creatinine level was non-significantly changed in comparison with non-infected camels (**Table, 4**). Theileriosis has deleterious influence on the liver function of the infected animals (Kawamura *et al.*, 1987). Theileriosis markedly affected the hematological and biochemical component of dromedary camels leading to disturbance of the liver, kidney and muscle functions (Ismael et al., 2014). An increase in the level of AST was attributed to damage to the skeletal or heart muscles, hepatic tissues and erythrocytes (Kataria and Bhatia, 1991). Also our study proved that there was increase in the mean level of urea of the parasitized camels as agree with Qarawi (1999) who explained that the increased level of urea was attributed to indirect damage of renal tissue and the existing of globin catabolites liberated due to loss of the hemoglobin by the reticulo-endothelial system via erythrophagocytosis process.

Histological alterations were in form of infiltration of Koch's blue bodies at lymphocytes, (**Fig. 2-a**) and multinucleated giant target cell with multiple prominent nucleoli (black arrow). Some lymphocytes contain basophilic parasitic organisms (koches blue bodies) (**Fig. 2-b**). Giemsa-stained section from lymph node of Thieleria infected camel showing koches blue bodies at lymphocyte (**Fig. 2-c**). While, liver showing distinct small and large vacuoles degeneration at the hepatocyte (**Fig. 2-d**). Also, there was necrosis at the lymphoid follicle and diffuse fatty degeneration at hepatic parenchyma and fibrosis in liver. Singh, et al. (2001) infected camels with blood parasites was leading to distinct histological deviations in the target organs involving liver and lymph nodes. It was distinguished with fatty degeneration of the hepatocytes with necrosis and depletion of the lymphoid follicles, besides infiltration of Koch's blue bodies at lymphocytes. Histological changes were attributed to progressive destruction of erythrocytes via reticulo-endothelial pahagocytosis resulted in hypoxic tissues and destructive damage to the related organs It was attributed to further destruction of erythrocytes through the reticulo-endothelial pahagocytosis resulted in tissues hypoxia and destructive damage to the target organs (Singh, et al., 2001).

Table (1): Percentage of prevalence of *Theileria* spp. recorded in camels examined throughout four different seasons.

Season	<i>Theileria</i> %
Autumn	8
Winter	2
Spring	1
Summer	4
Total %	15

Table (2): Hematological parameters including RBCs, WBCs, hemoglobin, PCV, MCH, and MCHC of non-infected camels and *Theileria* infected camels.

Parameters	Blood parameters					
	RBC's ($\times 10^6$)	WBCs ($\times 10^3$)	Hb. (gm/dl)	PCV (%)	MCH (Pg)	MCHC (%)
Non infected camels	11.0 \pm 0.8	11.2 \pm 0.5	14.3 \pm 1.1	32 \pm 0.9	13.0 \pm 1.1	44.6 \pm 0.4
<i>Theileria</i> infected camels	7.7 \pm 0.9*	10.6 \pm 0.3	11.5 \pm 0.6*	25.2 \pm 1.5*	14.8 \pm 0.5	45.7 \pm 0.5

* \rightarrow is referring to significant changes in comparison with non- infected camels when $P < 0.05$ %.

Table (3): Liver function tests of non-infected camels and *Theileria* infected camels.

Parameters	Liver Function Tests	
	AST (IU/l)	ALT (IU/l)
Non infected camels	69.6 \pm 2.5	11.0 \pm 1.0
<i>Theileria</i> infected camels	121.0 \pm 2.0*	11.0 \pm 2.1

* \rightarrow is referring to significant changes in comparison with non-infected camels when $P < 0.05$ %.

Table (4): Level of total protein, albumin, and globulin (gm/l) of non-infected camels and Theileria infected camels.

Parameters	Protein profile		
	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Non infected camels	9.53±2.1	3.14±0.5	6.4±1.7
Theileria infected camels	7.07±1.5	2.5±0.8	4.5±0.8

Table (5): Kidney function tests of non-infected camels and Theileria infected camels.

Parameters	Kidney function test	
	Urea (mg/dl)	Creatinine (mg/dl)
Non infected camels	25.0±1.5	1.1±0.0
Theileria infected camels	63.4±2.6*	1.0±0.2

*→ is referring to significant changes in comparison with non-infected camels when P<0.05 %.

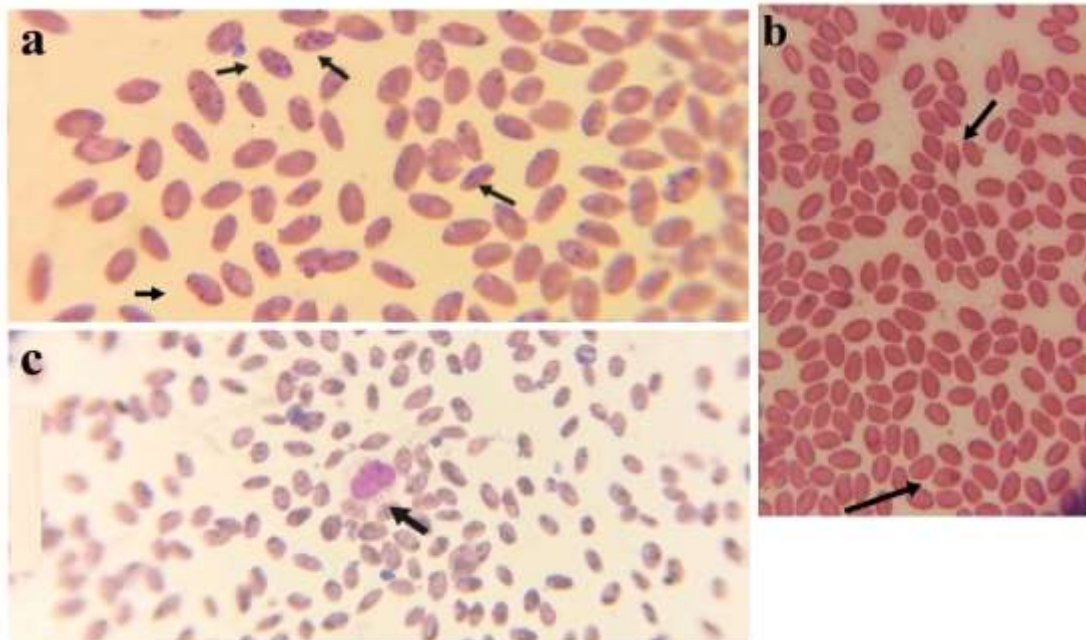


Fig. 1 (a-c): Giemsa-stained blood film showing *Theileria piroplasm* in cocci form within erythrocyte of camel (black arrow). Note also the hypochromic (red arrow) and macrocytic (blue arrow) erythrocytes (a), Giemsa-stained blood film from examined camel showing macrocyte cell (arrow) and *Theileria* infected RBCs in form of (signet ring) (b), Schizont of *Theileria* in lymphocytes of *Theileria piroplasm* with signet-ring within erythrocyte of camel (c). (Giemsa stain, X 1000)

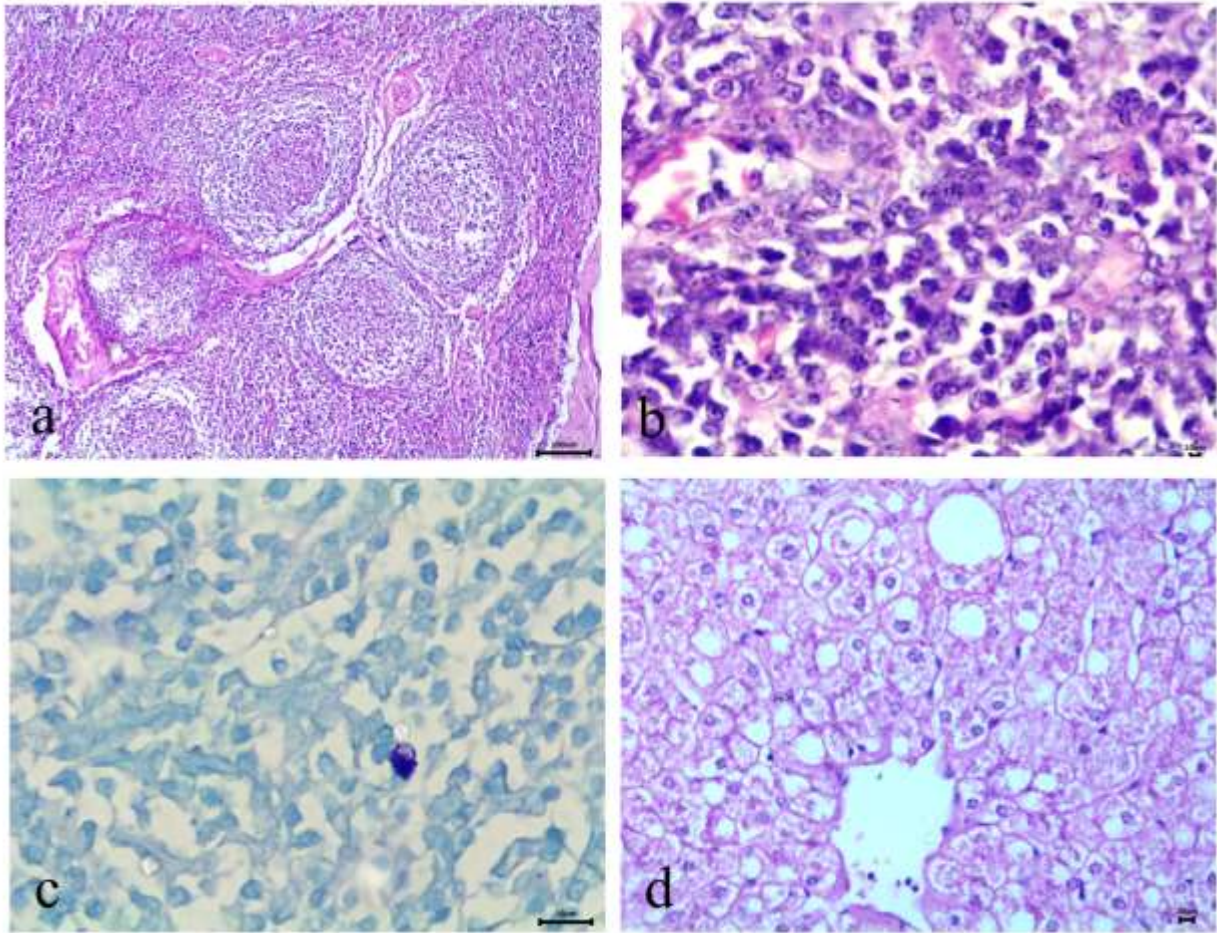


Fig. 2 (a-d): Photomicrograph from *Theileria* infected camel showing depletion and necrosis at the lymphoid follicle (a), multinucleated giant target cell with multiple prominent nucleoli (b), some lymphocytes contain basophilic parasitic organisms (Koch's blue bodies) (blue arrow) (b). Giemsa-stained section from lymph node of *Theileria* infected camel showing Koch's blue bodies at lymphocyte (c) Photomicrograph from *Theileria* infected camel showing distinct small and large vacuoles at hepatocyte (d). (H&E stain, bar=20 & 200 μ m)

4- Conclusion:

From the obtained results, it could be concluded that: Most of positive cases with Theileriosis are clinically health, this means that we face problematic to cure these infected animals as they become carriers of parasite and serve as reservoirs for transmission of infection to other animals. Even in these carrier animals, there were clinicopathological deteriorations involving hematological and biochemical alterations as well as histopathological changes on the target organs of the examined camels.

5- Recommendation

Screening and treatment of infected and carrier animals as well as control program of the vectors must be done specially during the seasons of vector-borne disease.

The authors declare that there is no conflict of interest.

6- References:

- Bacha W.J.J., Bacha L.M. (2000). Color Atlas of Veterinary Histology, 2nd ed., Philadelphia: Lippincott Williams & Wilkins.
- Bartels H., Bohmer M., Heirli C. (1972). Estimation of creatinine clearance in patients with unstable renal function. *Clin. Chim. Acta.* 37, 173.
- Bornstein S., Younan M. (2013). Significant veterinary research on the dromedary camels in Kenya: Past and present. *J. of Camelid Science.* 6, 1-48.
- Boulter N., Hall R. (2000). Immunity and vaccine development in the bovine theileriosis. *Adv Parasitol.* 44, 41-97.
- Doumas B.T., Watson W.A., Biggs H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clin., Chim., Acta.* 31, 87-96.
- El-Fayoumy M.M., Abou Elnga T.R., Abd El-Baky S.M.M., Abdou T.A. (2005). Prevalence of camel theileriosis and its vector tick in North Coast of Egypt. *J Egypt Vet Med Assoc.* 65, 291-302.
- El-Metenawy T.M. (2000). Prevalence of blood parasites among cattle at the central area of Saudi Arabia. *Vet Parasitol.* 87, 231-236.
- Farooq Z., Mushtaq S., Iqbal Z., Akhtar S. (2012). Parasitic helminths of domesticated and wild ruminants in Cholistan desert of Pakistan. *Int J Agric Biol.* 14, 63-68.
- Fawcett J.K., Scott J.E. (1960). Enzymatic determination of urea. *Journal of Clinical Pathology.* 13, 156-159.
- Feldman J.G., Zink I., Jain N.C. (2000). *Shalm's Veterinary Haematology*, 5th ed, Williams and Wilkins, Phildalphia and Baltimore.
- Gornal A.C., Bardawill C.J., David M.M. (1949). Colorimetric determination of total protein. *J. Biol., Chem.* 177, 751.
- Hamed M.I., Zaitoun A.M.A., El-Allawy T.A.A., Mourad M.I. (2011). Investigation of *Theileria camelensis* in camels infested by *Hyalomma dromedarii* ticks in Upper Egypt. *J Adv Vet Res.* 1, 4-7.
- Ismael A.B., Swelum A.A., Khalaf A.F., Abouheif M.A. (2014). Clinical, Haematological and Biochemical Alterations Associated with an Outbreak of Theileriosis in Dromedaries (*Camelus dromedarius*) in Saudi Arabia. *Pak Vet J.* 34(2), 209-213.
- Kataria N., Bhatia J.S. (1991). Activity of some enzymes in the serum of dromedary. *Camels Res. Vet Sci.* 51, 174-176.
- Kawamura M., Maede Y., Namioka S. (1987). Mitogenic responsi-bilities of lymphocytes in canine babesiosis and the effects of splenectomy on it. *Jpn. Vet. Res.* 35, 1 10.
- Mahrn O.M. (2004). Some studies on blood parasite in camels (*camelusdromedarus*) at Shalatin city, Red sea Governorate. *Assiut Vet. J.*; 50: 172-183.
- Mohammed E.S., Elshahawy I. (2018). The current prevalence of bovine babesiosis and theileriosis infection in Egypt, *J Bacteriol Mycol Open Access.* 6(6), 306-311.

- Mohandas N., An X. (2012). Malaria and Human Red Blood Cells. *Med Microbiol Immunol.* 201(4), 593–598.
- Mukhebi A.W., Perry B.D., Kruska R. (1992). Estimated economics of theileriosis control in Africa. *Prev Vet Med.* 12, 73-85.
- Nassar A.M. (1992): Theileria infection in camels (*Camelus dromedarius*) in Egypt. *Vet Parasitol.* 43, 147-149.
- Qarawi A.A. (1999). The chronobiological parameters changes as correlated to different trials of camelus dromedarius in Saudi Arabia. *J Camel Pract Res,* 6, 45-48.
- Radostits O.M., Gay C.C. Hinchcliff K.W., Constable P.D. (2007). Babesiosis. In: *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats.* 10th ed. Saunders/Elsevier, pp: 1483-1498.
- Reitman S., Frankel S. (1957). Colorimetric method for estimation of GOT/AST and GPT/ALT transaminases. *J. Amer. Clin. Pathol.* 28, 56-63.
- Salimabadi Y., Telmadarraiy Z., Vatandoost H., Chinikar S., Oshaghi M.A., Moradi M., Mirabzadeh-Ardakan E., Hekmati S., Nasiri A. (2010). Hard ticks on domestic ruminants and their seasonal population dynamics in Yazd province, Iran. *Iran J Arthropod-Borne Dis.* 4, 66-71.
- Singh A., Singh J., Grewal A., Brar S. (2001). Studies on some blood parameters of crossbred calves with experimental Theileria annulata infections. *Vet. Res. Commun.* 25, 289-300.
- Wagner G.G., Holman P., Waghela S. (2002). Babesiosis and heat water: threats without boundaries. *The Veterinary Clinics of North America Food Animal Practice.* 18(3), 417-430.