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# LABORATORY TESTS IN DROMEDARY CAMELS NATURALLY INFECTED WITH PIROPLASMS IN IRAN: STUDY AND REVIEW OF LITERATURE

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## ABSTRACT

Giemsa-stained peripheral blood smears from 114 apparently healthy dromedary camels aged 3 months to 18 years, kept in husbandaries of Yazd, Iran, during summer 2008 were examined microscopically. Piroplasm organisms were found in blood films of 18 camels (15.79%), all of them being adult and over 5 years old. The frequency of camel piroplasmosis in the study area is considerable. Serum samples obtained from jugular vein of the 110 adult camels were tested for 19 selected serum biochemical, enzymatic and hormonal parameters. There was no significant abnormality in those parameters (P>0.05). Piroplasmids do not seem to induce significant alteration in routine serum laboratory tests in naturally-infected dromedary camels. Previous publications about camel piroplasmosis, although a bit discordant, mainly support the idea of subclinical nature of this infection.

**Key words:** camel, Iran, laboratory tests, piroplasm, theileriosis

*Theileria* spp. are obligate intracellular tick-borne protozoan parasites belonging to the order piroplasmida, which cause world-wide infections in many domestic and wild animals.

Two species, namely *Theileria camelensis* and *Theileria dromedarii* have been reported from camel-breeding areas of the world (Chhabra and Sangwan, 2006). They are transmitted by common camel tick *Hyalomma dromedarii* (Kaufmann, 1996) provided that the erythrocytic piroplasm stage of the parasite is present. No microschizont stages have been yet described and thus, the taxonomic status of these parasites remains unclear (Gahlot, 2000; Coetzer and Justin, 2004). Recently *Theileria equi* and *Babesia cabali* were identified by PCR in clinically healthy Jordanian dromedaries (Qablan *et al,* 2012), but no piroplasmids were found in bactrian camels (Sloboda *et al,* 2010). *T. camelensis* is generally thought to be non-pathogenic, except in a few unpublished studies such as Hamidinejat *et al* (2008) who reported common signs of bovine tropical theileriosis in 2 camels and observed rapid recovery after buparvaquone treatment and its economic impact appears to be small (Boid *et al,* 1985). Biochemical alternations in serum of *Trypanosoma evansi* infected camels have been reported in the area of present study (Sazmand *et al,* 2011), and investigators working on Theileria have shown alterations in blood constituents and tissue lesions in naturally-infected cattle in Iran (Nazifi *et al,* 2008; Badiei *et al,* 2010), but little is known about the consequences of camel theileriosis and/or babesiosis worldwide. Nevertheless, Rao *et al* (1988) studied 5 biochemical and enzymatic parameters on sera of 14 naturally-infected camels.

The aim of this research was to investigate the effect of subclinical camel piroplasmosis on routine biochemical, enzymatic and thyroid hormonal parameters of dromedaries in Iran.

## Materials and Methods

**Study area:** The study was carried out in the Yazd province; an arid region in center of Yazd has a climate which mostly resembles dry desert climate with the mean temperatures of 30.67 and 8.36°C in summer and winter, respectively. Camels were kept by local farmers and were fed low quality diets containing mainly straw, barley and wilted grass.

**Sampling and investigation:** Blood samples were obtained from jugular vein. Giemsa-stained peripheral blood smears from 114 apparently

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healthy dromedaries aged 3 months up to 18 years were examined microscopically for the presence of piroplasms. Serum was also separated and stored at –20°C till analysis. The 19 measured serum parameters using commercial kits included glucose, urea, cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatin kinase, Ca, Mg, Na, Kp, albumin, total protein, tri-iodothyronine, thyroxine, and cortisol. Four camels below the age of 4 years (all negative for piroplasm) were omitted from the study, leaving 110 camels. A spectrophotometer (Shimadzu, model AA200, Japan) was used for biochemical colorimetric assays, flame photometry (digital flame analyser, model 2655–00, Cole–Pulmer Instrument, USA) for determination of sodium and potassium concentrations, and ELISA plate reader (Awareness, USA) for hormone assays.

**Statistical analysis:** Data were analysed by the SPSS (version 16, SPSS Inc., Chicago, IL, USA) software, using independent Student's t–test. P-value <0.05 was considered as statistically significant. Non-infected animals were considered as control for comparison of results.

## Results

Piroplasmid organisms were found in blood films of 18 camels (15.79%), all of them being adult and over 5 years old. There was no significant abnormality in parasitised camels (as compared with non-parasitised camels) in any of measured parameters (P >0.05). The main data are summarised in table 1.

## Discussion

According to the results, the frequency of camel piroplasmosis is considerable in the area (15.79%). Scientists from camel rearing parts of the world have reported different infection rates of camels to Theileria *spp*.; from those of Nassar (1992) who reported Theileria organisms in blood films of 60 (30%) of the 200 examined camels in Egypt, to Borji *et al* (2009) who did not find Theileria organisms in their epidemiologic study on 262 camels in eastern Iran (2009), and also Sloboda *et al* (2011) who found no positive animal in their survey on 70 Mongolian Bactrian camels. Recently Qablan *et al* (2011) in their survey on 100 camels in Jordan found 10 of them to be infected with equine piroplasms (10%) by PCR assay, although neither Theileria nor Babesia stages were detected in erythrocytes during microscopic examination. They identified 6 of them harboring *Babesia cabali* and other 4 *Theileria equi* (2011). Salim Abadi *et al* (2010) noticed that *Hyalomma dromedarii* (vector of camel theileriosis) was the predominant tick species and accounted for 55.92% of the 583 collected

**Table 1.** The mean (±SEM) concentration of serum constituents of priroplasm infected and non–infected dromedary camels.

| Parameter | Method | Units | Infected camels (n=18) | Non-infected camels (n=92) |
|---|---|---|---|---|
| Glucose | enzymatic (GOD-PAP) | mmol/L | 3.31±0.38 | 3.40±0.14 |
| Urea | enzymatic (urease) | mmol/L | 10.25±0.96 | 9.56±0.34 |
| Cholesterol | enzymatic (CHOD-PAP) | mmol/L | 0.86±0.97 | 0.87±0.04 |
| Triglyceride | enzymatic (GPO-PAP) | mmol/L | 0.75±0.24 | 0.49±0.05 |
| ALT | enzymatic (IFCC) | IU/L | 20.72±3.34 | 16.41±1.01 |
| AST | enzymatic (IFCC) | IU/L | 93.55±4.87 | 114.55±8.62 |
| ALP | enzymatic (DGKC) | IU/L | 104.22±8.67 | 116.48±8.96 |
| LDH | enzymatic (DGKC) | IU/L | 438.78±40.72 | 513.79±40.61 |
| CK | enzymatic (IFCC/DGKC) | IU/L | 102.72±21.15 | 100.99±17.99 |
| Ca | cresol phethalein complex | mmol/L | 2.55±0.06 | 2.52±0.03 |
| Mg | xylidyl blue | mmol/L | 1.13±0.03 | 1.07±0.02 |
| Na | flame photometry | mEq/L | 167.11±3.09 | 167.48±1.31 |
| K | flame photometry | mEq/L | 6.69±0.26 | 6.25±0.11 |
| P | ammonium molybdate | mmol/L | 2.11±0.12 | 1.97±0.05 |
| Albumin | bromcresol green | g/dL | 3.71±0.13 | 3.73±0.05 |
| Total protein | biuret | g/dL | 7.59±0.16 | 7.40±0.08 |
| T3 | ELISA | nmol/L | 4.90±0.81 | 4.87±0.37 |
| T4 | ELISA | nmol/L | 172.30±23.68 | 152.57±8.25 |
| Cortisol | ELISA | nmol/L | 9.13±1.95 | 10.57±0.98 |

**Table 2.** Changes induced by bovine theileriosis in serum constituents of infected animals.

| Parameter | Change | Given explanation | Reference |
|---|---|---|---|
| Glucose | NS* ↓ | Whole milk fed young animals | Sandhu *et al,* 1988 |
| | ↓ | utilization of glucose by the parasites, and hepatic dysfunction | Yadav and Sharma, 1986 |
| Urea | ↑ | Focal to diffuse coagulative histopathologic changes, necrosis, severe damage to collecting tubules, haemorrhages and lymphocytic aggregations | Sandhu *et al,* 1998 |
| Cholesterol and Triglycerides | ↓ | Anorexia associated with the high rise of temperature, and diarrhoea causing impaired absorption of fatty acids | Singh *et al,* 2001 |
| | NS* ↓ | Whole milk fed young animals | Sandhu *et al,* 1998 |
| | ↑ | Injury to the liver | Yadav and Sharma, 1986 |
| | ND** | – | Omer *et al,* 2003 |
| ALT and ALT | ↑ | Severe damage to hepatobilliary system due to hypoxia resulting from anaemia and jaundice | Sandhu *et al,* 1998 |
| | ↑ | Cardiac infarcts and severe liver damage (AST only) | Omer *et al,* 2003 |
| | ↑ | Muscular trauma as a result of prolonged recumbency | Izzo *et al,* 2010 |
| | | Hypoxia-induced hepatopathy and cholestasis (AST only) | |
| ALP | ↑ | Liver damage | Yadav and Sharma 1986; Sandhu *et al,* 1998; Omer *et al,* 2003; Badiei *et al,* 2010 |
| LDH | ↑ | Vascular thrombosis, haemorrhage, tissue necrosis and oedema especially in liver and kidney | Nazifi *et al,* 2008 |
| CK | ↑ | Muscular damage due to the anaemic condition and prolonged recumbency | Sandhu *et al,* 1998 |
| Ca | ↓ | Hypoalbuminemia, hypomaegnesaemia and kidney damage | Yadav and Sharma, 1986; Singh *et al,* 2001; Omer *et al,* 2003 |
| Mg | ↓ | Injury to the liver and other organs | Yadav and Sharma, 1986 |
| | ↓ | Diarrhoea and renal wasting | Omer *et al,* 2003 |
| na | ND** | - | Yadav and Sharma, 1986 |
| K | ↓ | Dehydration and kidney damage | Yadav and Sharma, 1986 |
| | ↓ | Inappetence, diarrhoea and hypomgnesaemia | Omer *et al,* 2003 |
| P | ↑ | Renal tubular defects | Yadav and Sharma, 1986; Sandhu *et al,* 1998; Omer *et al,* 2003 |
| | ↓ | Diarrhoea and renal wasting | |
| Albumin and Total protein | ↓ | Reduced synthesis because of the effect on liver, anorexia and diarrhoea | Singh *et al,* 2001; Omer *et al,* 2003; Badiei *et al,* 2010 |
| | ↓ | (Only total protein) | Yadav and Sharma, 1986 |
| T3 and T4 | ↓ | Liver failure | Badiei *et al,* 2010 |
| Cortisol | NS* ↓ | Thyroid hormones had no role in the regulation of HPA axis in the process of the disease | Badiei *et al,* 2010 |

↑: Increase; ↓: Decrease; *NS: Not significant; **ND: No difference

hard ticks in their study on domestic animals in the same area as our study. In the latter study vectors of babesiosis were also reported. Considering the fact that *H. dromedarii* also parasitises cattle and is a vector of bovine *T. annulata*, it is postulated by some that Theileria-like organisms found in the camel blood were of bovine origin with short survival in the abnormal host (Gahlot, 2000). However recent findings by Qablan *et al* (2012) suggest horses and canines may contribute in circulation of camel piroplasmosis.

In our work there was no significant abnormality in the measured parameters in the infected subjects except a mild reduction in AST levels (P>0.05). The only available study on serum parameters in camel piroplasmosis is that of Rao

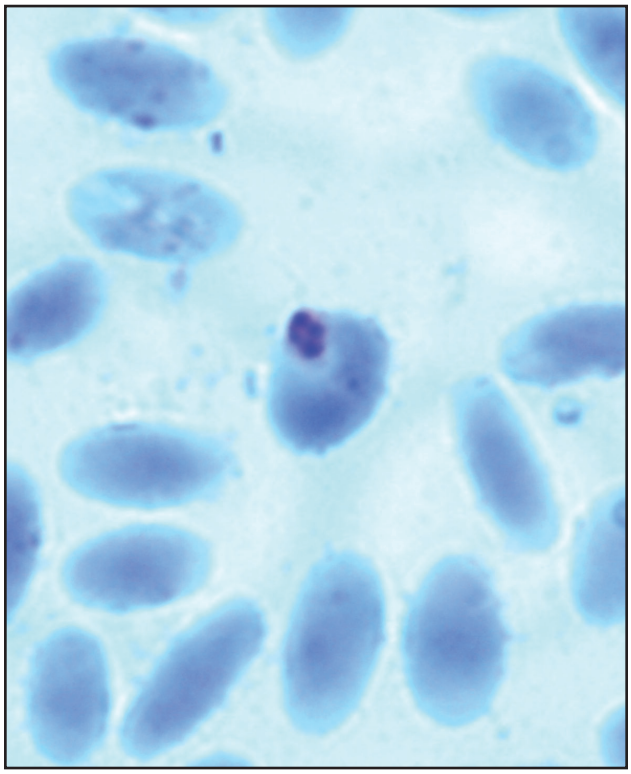

**Fig 1.** Photomicrograph of piraplasm in a camel RBC (Peripheral blood, Giemsa staining, ×1000)

*et al* (1988) who measured serum total protein, glucose, cholesterol, acid phosphatase and ALP of 14 asymptomatic camels infected with *T. dromedarii*. The serum ALP activity was lower and the serum glucose higher in infected animals. The values for total protein, cholesterol and acid phosphatase were not significantly different in infected and uninfected individuals.

The rest of our knowledge about serum analyte changes induced by theileriosis comes from studies on other animals (mostly cattle), which are summarised in table 2.

It seems that the different results in laboratory tests on some parameters may be due to different study areas, circumstances and methods in those studies, especially in selection of animals and the stage in which they had been sampled. However, the remarkable common explanation in most of the noticed abnormalities is the tissue hypoxia or necrosis that is a consequence of severe anemia or haemolysed RBCs blocking the microcirculation.

Since all of the camels in our study were asymptomatic, the observation of normal lab tests in them is justified. So, it seems that the above-mentioned routine laboratory tests in asymptomatic camels naturally-infected with piroplasm do not seem to help in its prognosis or treatment. Regarding considerable prevalence of piroplasmosis in camels of this region, prevention of the disease by controlling ticks seems necessary.

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