

Anaplasmosis among Camels in Iran and Observation of Abnormalities in Infected Blood Films

Khosro Ghazvinian, Touba Khodaiean

Abstract—*Anaplasma* organisms are obligatory intracellular bacteria belonging to the order Rickettsiales, family Anaplasmataceae. This disease is distributed around the globe and infected ticks are the most important vectors in anaplasmosis transmission. There is a little information about anaplasmosis in camels. This research investigated the blood films of 35 (20 male, 15 female) camels randomly selected from a flock of 150 camels. Samples were stained with Giemsa and *Anaplasma* sp. organisms were observed in six out of 35 (17.14 %) blood films. There were also some changes in Diff-Quick and morphology of leukocytes. No significant difference between male and female camels was observed ($P>0.05$). According to the results anaplasmosis is presented among camels in Iran.

Keywords—Anaplasma, camel, anaplasmosis, Iran.

I. INTRODUCTION

CAMELS are growing in demand and popularity for a different reason including the transportation of goods and humans, as well as for their milk and meat, wool and hides, and even their dung, which is used for fuel. They are in the class Mammalia, order Artiodactyla, suborder Tylopoda.

Camels re-chew their food but their stomachs are different from ruminants. These animals can survive in arid and semi-arid regions [15]. Camel meat fat is less than beef and camel wool is one of the world's most expensive animal fibers. Camel leather and milk are considerable products nowadays [15]. Some countries try to make camel's hybrid for increasing these products but infectious diseases can directly and indirectly influence on these products and their quality.

Anaplasma spp. are obligatory intracellular Gram-negative bacteria, order Rickettsiales, family Anaplasmataceae can infect camels [9]. In this order, organisms which replicate only in host cells. The application of ribosomal RNA sequencing and other methods has led to the reclassification of these organisms [9]. In Anaplasmataceae, the organisms don't have cell walls, they cannot be cultured *in vitro* [9]. *Anaplasma ovis*, *A. marginale* and *A. centrale* are erythrocytic *Anaplasma* species. *A. bovis* and *A. phagocytophilum* infect leukocytes and *A. platys* can be detected in platelets [9]. Anaplasmosis is caused by intraerythrocytic *Anaplasma* organisms among ruminants. *A. marginale* has been reported from cattle and wild ruminants, *A. centrale* can cause mild anemia in cattle. *A. ovis* and *A. marginale* have been reported among sheep and goats

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[9], [11]. This disease is distributed worldwide and ticks are the most important vectors in anaplasmosis [4], [5], [9]. The one-host tick *Boophilus* spp. and *Dermacentor* spp. are the most important vectors. *Rhipicephalus* spp. are also important in Australia [11]. Anaplasmosis transmission can also occur transplacentally and mechanically by biting flies; such as Tabanid flies [11]. The most dominant symptoms are fever, depression, weight loss, anemia, abortion, pale icteric mucosa, and sometimes death [5], [9], [15].

Recovered animals from acute anaplasmosis have a persistent infection by repetitive cycles of rickettsemia [11]. There are some diagnostic techniques for anaplasmosis such as observation of the organisms in the stained blood films, serological and molecular tests [9]. The disease must be differentiated from other causes of hemolytic anemia. The aim of this study is to understand whether *Anaplasma* infection presents in camels in Iran.

II. MATERIALS AND METHODS

The blood films of 35 camels (20 male, 15 female) were selected randomly from a flock of 150 camels. The flock was in Khorasan Razavi province, northeast of Iran. Blood films were fixed with methanol and stained with Giemsa, then observed by light microscope. The significant difference between male and female infected camels was analyzed by Chi-square test, the SPSS software.

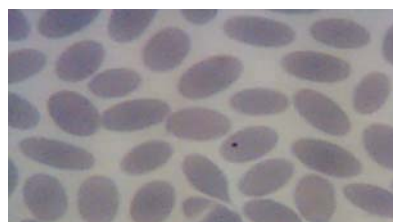


Fig. 1 *Anaplasma* organism in a camel's reticulocyte. 200X, Stained by Giemsa



Fig. 2 Reactive lymphocyte with increased cytoplasm basophilia. 400X, stained by Giemsa

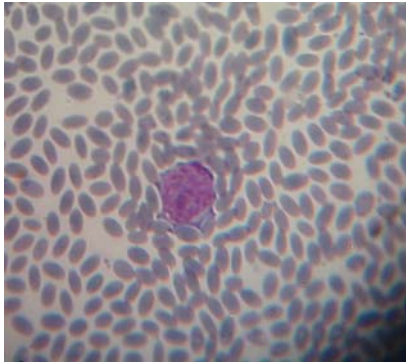


Fig. 3 Reactive lymphocyte with irregular cytoplasm 100X, stained by Giemsa

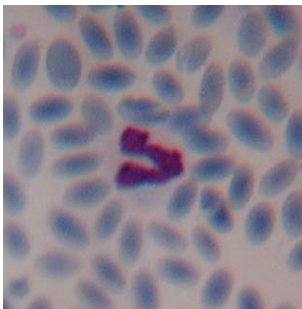


Fig. 4 Immature neutrophil (band cell), 200X, stained by Giemsa

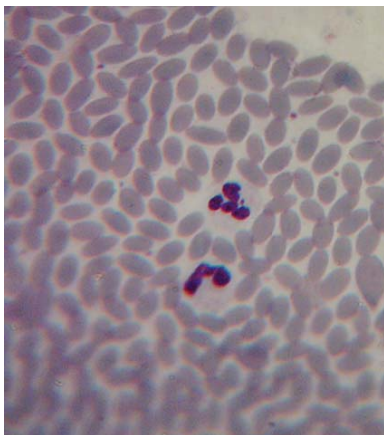


Fig. 5 Mature neutrophil (top) and a band cell (bottom), 150X, stained by Giemsa

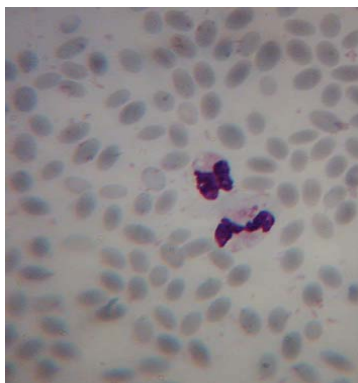


Fig. 6 Neutrophils with toxic change, 100X, stained by Giemsa

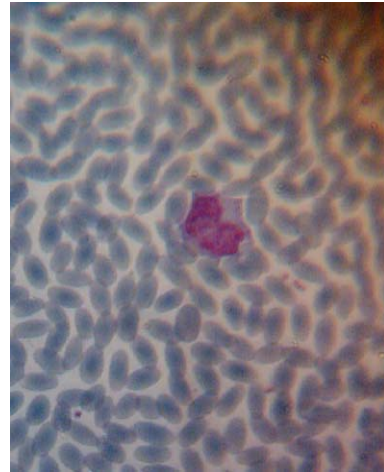


Fig. 7 Monocyte in camel's blood film, 150X, stained by Giemsa

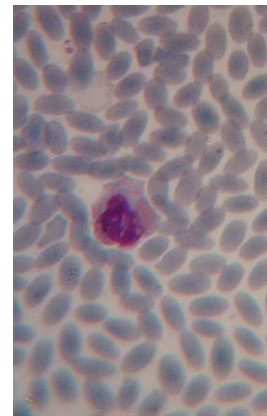


Fig. 8 Eosinophil in camel's blood film, 200X, stained by Giemsa

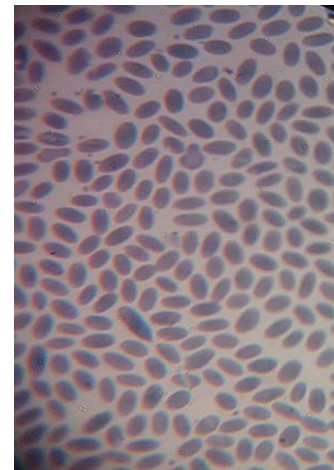


Fig. 9 Anisocytosis, 100X, Stained by Giemsa

III. RESULTS

Anaplasma organisms were observed in 6 (4 male, 2 female) out of 35 (17.14%) blood films (Fig. 1). There isn't any significant difference between male and female camels ($P>0.05$). In Diff-Quick; neutropenia with toxic changes, lymphopenia and reactive lymphocytosis (Figs. 2-6) were

observed in all positive samples. Granular lymphocytosis was also observed in 3 *Anaplasma* infected samples. The other leukocyte count is normal (Figs. 7 and 8). Anisocytosis with a mild poikilocytosis was the only detected change among reticulocytes (Figs. 9-11).

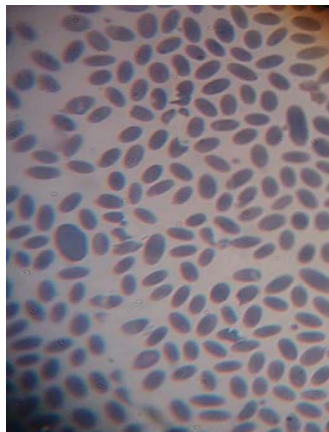


Fig. 10 Anisocytosis, note the hyper chromatophilic macrocytes, 100X, stained by Giemsa

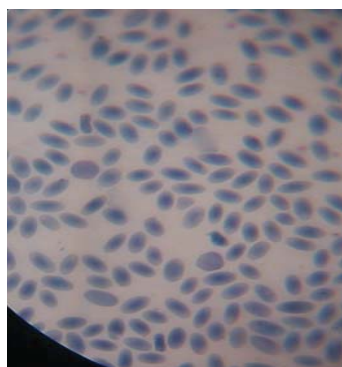


Fig. 11 Anisocytosis and mild Poikilocytosis, note the round reticulocytes, 100X, stained by Giemsa

IV. DISCUSSION

The information about the camels' infectious diseases such as anaplasmosis is too little all around the world. In Somalia the prevalence of anaplasmosis among camels has been reported about 40% [8]. *A. marginale* antibody has been found in 10.7% of examined camels in Nigeria [2], [10].

A. marginale has been detected in 84% Arabian one-humped camels in Iraq [1]. *Anaplasma* and *Babesia* were in 21.5% of Nigerian camels [6]. A 7-year male camel in India was infected with *Anaplasma* sp. with the loss of appetite and icteric mucosa [13]. The *Anaplasma* infected blood of a camel could be successfully transmitted to a cattle [15]. It is proposed that the infective species in camel anaplasmosis is *A. marginale* but the accurate diagnosis must be made by molecular examination. There is no information of *Anaplasma* potential vectors in camels but *Anaplasma platy* DNA was detected in *Rhipicephalus* sp. on infected Bactrian camels in China [7].

Hemolytic anemia occurs as a result of increased

reticulocyte destruction. Hemolytic anemia is generally regenerative and Anisocytosis is usually present in regenerative anemia [3], [12]. Anisocytosis is an erythrocyte size variation and it occurs when the different population of reticulocytes is present [3].

Increased hyper chromatophilic reticulocytes usually present in regenerative anemia. When the anemia degree is severe, macro reticulocytes may be released into the blood. These are immature reticulocytes which are twice bigger than normal size [3].

Basophilic stipplings are blue-staining inclusions in reticulocytes which are another sign of regenerative anemia [3] has also been observed in ovine anaplasmosis in Iran [10] but it was not observed in any cases of these infected camels. In leukocyte abnormalities; neutropenia (decreased numbers of neutrophils) can occur in inflammatory conditions. The bone marrow storage pool is depleted and there is not enough time for granulopoiesis [12]. Toxic changes such as an increase in cytoplasmic foamy vacuoles are often present in these conditions [12]. Endogenous glucocorticoids in animals can cause lymphopenia. Glucocorticoids sequester lymphocytes in lymphoid organs and stimulate the apoptosis of sensitive lymphocytes [3]. Natural killer cells or cytotoxic T lymphocytes can appear as granular lymphocytes. These kinds of lymphocytes may increase in inflammatory disorders [12]. Different antigens can stimulate lymphocytes proliferation. These lymphocytes are bigger than normal lymphocytes and their cytoplasm is more basophilic. These antigenically stimulated lymphocytes called "reactive lymphocytes", "transformed lymphocytes" and "immunocytes". Some of the reactive lymphocytes like monocytes are with basophilic cytoplasm [3].

In Diff-Quick in ruminants, neutrophilia in acute bacterial infections is less common and neutropenia is observed more commonly [14]. Reactive and granular lymphocytosis remain in peripheral lymphoid tissues, and very few of them may enter the circulation [12]. Lymphocytosis and neutropenia were reported among *Anaplasma* infected Arabian one-humped camels in Iraq [1]. According to the results neutropenia with toxic changes, reactive lymphocytosis and anisocytosis were observed in all *Anaplasma* infected samples. Granular lymphocytes were observed in 3 positive samples. Anaplasmosis has economic importance; death and abortion among infected animals have been reported. Loss of production in recovered and sick animals and preventive measures for tick control are cost associated aspects of the disease [11]. The average temperature and arid areas are increasing in Iran therefore growing of camels are cost beneficial but there is little knowledge about their diseases. This research showed that anaplasmosis presents among camels in Iran, therefore further researches are necessary to understand its pathogenicity, epidemiology and its potential vectors.

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