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***Dipetalonema evansi* Infection in Camels of Iran's Central Area**

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Abstract: Totally 294 dromedary camels of different ages and both sexes slaughtered at slaughterhouses in Yazd, Isfahan and Kerman provinces were inspected for infection with *Dipetalonema evansi*. Blood smears of all camels and carcasses of 125 of them (100 from Isfahan and 25 from Yazd) were studied for larva and adult forms of the parasite. Microfilariae were found in peripheral blood smears of 38 out of 294 (12.92%) tested camels, while 20 out of 125 camels (13.89%) harbored *D. evansi* adult worms in at least one region in their testicle, epididymis, spermatic cord, lung and heart. Two of infected males had adult forms of the parasite in all studied organs simultaneously. Pathological study of infected tissues revealed sections of parasite, severe acute and chronic inflammation, fibrosis and atrophy. *D. evansi* is endemic and constitutes an important health problem to camels in Iran's central desert, resulting in impaired working capacity and lowered productivity.

Key words: *Dipetalonema evansi*, prevalence, pathology, camel

INTRODUCTION

Dipetalonema evansi is a filarial nematode which specifically affects camels and lives in the heart, in hepatic, pulmonary and spermatic arteries, lymph nodes and lymph vessels (Nagaty 1947, Dakkak and Ouhelli, 1987). This parasite has been reported from almost all camel rearing areas in the world (Wernery and Kaaden, 2002). Despite common prevalence in Saudi Arabia, Iran and Egypt, there seems to be a few reports from India (Pathak and Chhabra, 2010). The reported estimates of prevalence in different countries ranges from 4% in adult camels to 47.5% in camels less than one year old (Muhammad and Athar, 2000). Manifestations of dipetalonemiasis include weakness, loss of appetite, pale mucous membrane, orchitis, aneurysms in the spermatic cord, arteriosclerosis and heart failure (Kaufmann, 1996; Chhabra and Gupta, 2006). Microfilariae feed on blood in peripheral blood and a marked decrease in hemoglobin level and eosinophilia are seen in blood examination (Fassi-Fehri, 1987; Muhammad *et al.*, 2004). Orchitis and epididymitis due to *D. evansi* infestation have also been reported in camels, with an incidence of 2.3-10% in wet regions (Tibary *et al.*, 2006). Scientists from Iran have

studied dipetalonemiasis of camels from different geographical regions and reported prevalence of up to 50% (Mirzayans and Halim, 1980; Moghaddar *et al.*, 1992; Mowlavi *et al.*, 1997; Zarfi-Fard and Fesharaki, 2000; Oryan *et al.*, 2008; Borji *et al.*, 2009; Fard *et al.*, 2011). As the parasitic diseases are the major cause of impaired milk and meat production in animals and have adverse impact on health, productivity and working capacity of camels (Shafqaat *et al.*, 2004) and considering the fact that camel is an important multipurpose animal in Iran (Sazmand *et al.*, 2012), the present study was conducted to investigate prevalence and distribution pattern of camel *Dipetalonema evansi* infection in central area of Iran.

MATERIALS AND METHODS

The research was carried out in arid areas of Iran. Three central provinces of Yazd, Isfahan and Kerman were selected. These regions are adjacent to the Iran's central desert. One hundred and fifty apparently healthy dromedaries of both sexes and different ages were selected randomly and jugular vein blood was withdrawn, mixed with EDTA and kept in refrigerator (2-8°C) until examination before 24 h. Diagnosis was made by

examination of wet or stained thin blood films or by concentration of microfilaria by Knott technique to detect *Dipetalonema evansi* microfilariae (Anonymous, 1977; Soulsby, 1982).

In a second study on 144 dromedaries (65 males and 79 females) slaughtered in Yazd, Najaf Abad and Rafsanjan abattoirs, we carried-out careful clinical examination before slaughter and also gross inspection for presence of *D. evansi* after slaughter. Specimens of blood, testis, epididymis, spermatic cord, trachea, bronchi, lung and also heart of them were obtained. Blood smears were prepared similar to the above-mentioned study. Infected and suspicious organs were transferred into Normal Saline Solution (NSS) for washing, centrifugation and subsequent collection. The recovered helminthes were examined by light microscopy in fresh state and also as fixed in 70% ethanol followed by lactophenol treatment, after which they were counted and measured. Identification and measurements of this worm were conducted according to keys described by Soulsby (1982). Tissue samples of infected testes were fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, embedded in paraffin, sectioned at 5 µm thicknesses, stained with hematoxylin and eosin and studied with a routine light microscope by a parasitologist and a pathologist.

The obtained data were analyzed using SPSS software for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA). To compare relative frequency of infection between different age groups of camels, Chi-square test was used. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Sheathed microfilariae were found in peripheral blood smears of 38 (12.92%) out of 294 tested camels. *D. evansi* adult worms were recovered from at least one of the below organs in 20 (13.89%) camels: epididymis, testis, spermatic veins, heart and lungs (Table 1). The maximum adult nematode infection rate was seen in testes. The overall infection rate of male camels with either microfilariae or adult worms was significantly higher than females ($p = 0.0001$). In two tested males, simultaneous adult *D. evansi* worms' infection in three major organs of testis, heart and lungs was observed.

Table 1: Distribution of affected organs in two sexes

Affected organs sex	(No. %)			
	Testes	Hearts	Lungs and air passages	Total filariae
Male (n = 65)	23.08 (15/65)	1.54 (1/65)	1.54 (1/65)	23.08 (15/65)
Female (n = 79)	0	2.53 (2/79)	3.8 (3/79)	3.8 (3/79)
Total (n = 144)	23.08 (15/65)	2.08 (3/144)	2.78 (4/144)	13.89 (20/144)

D. evansi mature nematodes were recovered from either of the arteries of the spermatic cord, testicle, epididymis, lungs and heart of infected camels. The worms recovered from organs had different lengths, females being larger (14.8-21 cm) than males (8.5-10.5 cm). The largest and smallest spicules were 550-700 and 160-180 µm long, respectively. Microfilaria measured 200-300 µm in length.

The main pathologic findings in the studied tissues included sections of parasite, severe acute and chronic inflammation, fibrosis and atrophy. More detailed microscopic evaluation of the testis showed severe atrophy of the seminiferous tubules, thickening of their basement membrane, Sertoli cell-only syndrome (total germ cell aplasia) and sloughing of germ cells in some tubules. Only rare spermatozoa could be found in most of the affected testes. Usually, the interstitial Leydig cells were preserved. The surrounding tissues displayed cross sections of the worms, profound infiltration of eosinophils, lymphocytes, some histiocytes and some neutrophils. Peripheral to this severe inflammation, there was usually marked fibrosis and disarrangement of the tissues. Blood vessels in the affected regions showed prominent endothelial cells. There were some fresh and/or old hemorrhagic areas. Granuloma formation was occasionally detected. In the case of long-standing tissue invasions by the worm, hyalinization or calcification was found as a clue to its chronicity. Spermatic cord and epididymis also manifested similar pathologic findings (Fig. 1-3).

The prevalence rate of *Dipetalonema evansi* microfilaria in blood in the present study was 12.92%. Researchers from different geographical regions of Iran have reported various infection rates of camels with microfilarial form of this worm. Fard *et al.* (2011) and

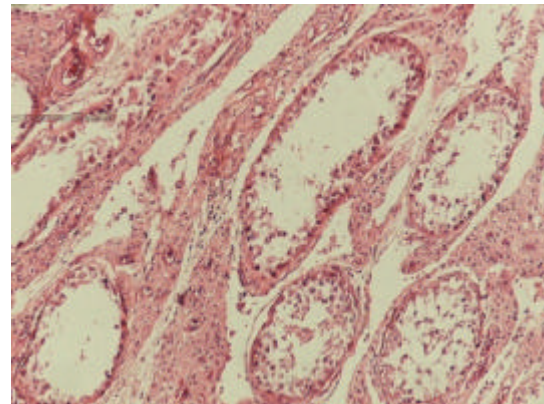


Fig. 1: *D. evansi* adult parasites in the testicular tissue (Hematoxyline and Eosin staining ×100)

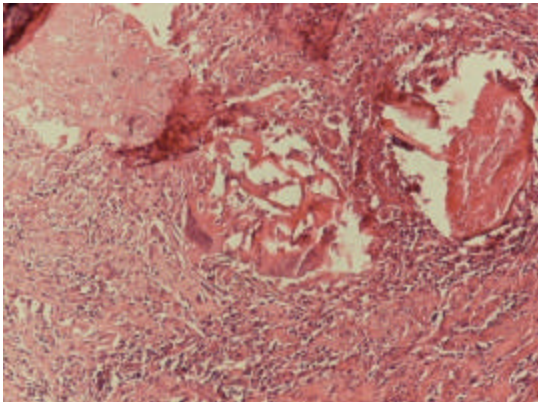


Fig. 2: Chronic granulomatous inflammation with multinucleated giant cells around the worms (Hematoxyline and Eosin staining×100)

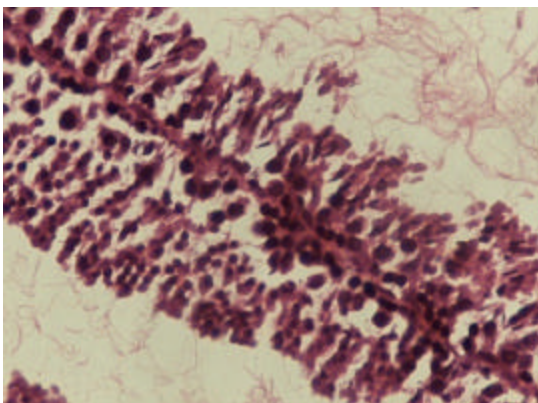


Fig. 3: Sertoli cell-only syndrome in the affected camel testis (Hematoxyline and Eosin staining×400)

Borji *et al.* (2009) reported lower rates down to 2-3 and 5.34%, respectively. However, higher rates of 20.7, 28.1 and 46.7% are reported from camels in other parts of the country (Rahbari and Bazargani, 1995; Mowlavi *et al.*, 1997; Oryan *et al.*, 2008). The difference in reported rates of infection may be due to the difference in management systems of the dromedaries.

Although the rate of circulating microfilarial infection in this study was higher among female than male dromedaries (20.16 vs. 7.64%), but it was not statistically significant. Similarly (Borji *et al.*, 2009; Fard *et al.*, 2011) found that infection rate of *D. evansi* was not significantly different between male and female camels.

In addition, the prevalence rate was highest in animals aged below 5 years. Similarly, Rahbari and Bazargani (1995) observed a significant inverse relationship between host age and prevalence of

infection. It may be due to decrease in susceptibility with advancing age (Muhammad and Athar, 2000). In contrast, (Borji *et al.*, 2009; Fard *et al.*, 2011) reported that the infection rate increased with age, although no significant relationship between the rate of infection with *D. evansi* and age was observed.

In our study, 15.28% of tested camels showed adult worms of *D. evansi* at necropsy. Infection was higher in males (17/22) than females (5/22). In a study by Fard *et al.* (2011) on 309 camels slaughtered at Kerman slaughterhouse in Iran, 49 (15.85%) of them from different ages and both sexes were infected with adult forms of *D. evansi* in either of their testicle, epididymis, spermatic cord, or lungs. Most of the other researchers in Iran have studied infection in one sex (males) or one organ and reported various rates of adult worm infection. Oryan *et al.* (2008) and Borji *et al.* (2009) studied only male dromedaries and reported that 20.7 and 5.34% of their tested camels were infected with adult worms, respectively. Mowlavi *et al.* (1997) found that half of the Iranian male dromedaries (50%) in Najaf Abad and Yazd abattoirs harbor testicular *D. evansi*. Moghaddar *et al.* (1992) however, reported adult worms in the lungs of 17.5% of 40 tested camels in Shiraz province, Iran.

Histopathologic findings in the present study are almost in agreement with previous studies.

Results of this study showed that dromedaries in central part of Iran frequently suffer from low grade infections with *D. evansi*, which indicates the necessity of more research on outstanding reproduction, tissue migration, vectors and host-parasite interactions of this filarial nematode parasite.

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