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ORIGINAL ARTICLE

Histopathologic and histomorphometric evaluation of *Dipetalonema evansi* infection in camel testicular tissue

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Abstract Camels are important sources of milk, meat, wool and leather, and are widely used in transportation in arid and semi-arid areas. But their illnesses, especially parasitic diseases, have not been taken into consideration. The *Dipetalonema evansi* microfilariae are in the blood. Adult nematode is only dedicated to camels and disrupts spermatic arteries, lung arteries, right atrium, and testicles. This study was carried out on testicular samples of camels infected with *D. evansi* referred from slaughterhouse. In each of the control and contaminated groups, 5 samples were examined. In this study, in addition to the qualitative description of parasite histopathologic lesions, the spermatogenesis process was evaluated quantitatively including spermatogenesis process, diameter of the seminiferous tubules and Johnsen ranking and compared with the control group. Histopathological examination of infected testis with *D. evansi* showed lumen obstruction of testicular blood vessels by parasites, hypertrophy of blood vessels, degenerative and necrosis changes in the tubules, decreased spermatogenetic activity, increased interstitial space tubules, destruction of the spermatogenic cells. Also, there was a significant difference in the control and contaminated groups in the parameters of spermatogenesis, diameter of the seminiferous tubules and Johnsen score.

Keywords *Dipetalonema evansi* · Microfilariae · Histopatology · Histomorphometry

Introduction

The camel is the most important domesticated animal in tropical regions due to milk and meat productions along with working purposes. In Iran, the history of camel goes back to the Achaemenid kings at 530 BC, where some subsidiary nations brought this animal as a gift to the king (Allen 2005). Camels live in arid and semi-arid areas of Iran including deserts of Khorasan, Sistan and Baluchestan, Kerman, and Yazd provinces and play an important role in economy of these regions. Various factors can affect camel products such as milk, meat and wool. One of the main reasons for reduction of milk and meat production is parasitic infections that have adverse effects on health condition of camels (Shafqaat et al. 2004). *Dipetalonema evansi* (*D. evansi*) is a nematode of filarial class in camels that develops in the heart, and hepatic, pulmonary and spermatic arteries as well as mesenteric lymph nodes and lymph vessels (Elamin et al. 1993; Oryan et al. 2008). The male and female nematodes have 7.5–8.0 cm and 14–21.5 cm length respectively. Microfilariae with 200–315 µm length can be detected in the peripheral blood (Nagaty 1947). Possible vectors for this parasite are different species of *Aedes* mosquitoes. Mosquitoes ingest microfilariae during nutrition from blood of infected dromedary. Then, parasites move to the chest muscles of mosquitoes, where they continue to develop. After 10 days, presented larvae in mosquito's proboscis have ability for

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causing infection in a new host (Duvallet and Boireau 2015). Mild infections are not diagnosed. In severe infections, the symptoms are cachexia, sometimes orchitis, nervous manifestations and probably death (Chhabra and Gupta 2006).

The presence of *D. evansi* has been reported in camels in Southern Iran and Shiraz Province as 17.5% and 28.1% respectively (Rahbari and Bazargani 1995). Based on a series of different previous study, prevalence of *D. evansi* microfilariae in blood samples was 0.88% to 46.7% (Sazmand and Joachim 2017).

One of the main features of food animal rearing is reproductive efficacy. In many tropical regions of the Iran, the reproductive management of camels is traditional and the owner's camels handle the mating process in the rutting season. In fact, the reproductive performance of dromedary camels is low due to the short duration of breeding season, difficult sperm collection, and late to reach sexual maturity in comparison with other farm animals (Skidmore 2003). On the other hand, low fertility of male animals in such natural mating reproductive management can led to great pregnancy failures. Testicular degeneration induced by infections, chemical and environmental factors is one of the main reasons for decreasing and disturbing spermatogenesis processes and subsequent low sperm concentration and quality. It was shown that caused filariasis by *D. evansi* could induce orchitis and spermatic cord hematomas (Hemeida et al. 1985; Moghaddar et al. 1992). In the present study, the histopathologic and histomorphometric changes of infected testes with *D. evansi* are investigated in *Camelus dromedaries.*

Materials and methods

Animals and study design

The study was performed on 5 infected testes to *D. evansi* in order to investigation of spermatogenic parameters and compare with 5 none infected testicular tissues. The infected samples were obtained from the previous publication (Nourollahi et al. 2011). Bilateral spermatic cords, testicles and epididymis of all samples were inspected grossly for finding of mature form of *D. evansi*. The recovered *D. evansi* were kept in the normal saline solution, counted and measured. They were examined in fresh state, fixed in the 70% ethanol followed by lactophenol treatment, then examined with a light microscopy.

Testicular samples from each group were taken and fixed in the 10% neutral buffered formalin. After fixation, the tissue samples were processed according to the routine histopathologic technique. Tissue sections in 5 μm thickness were prepared and stained with hematoxylin–eosin and studied with a light microscope (Nikon, Digital Sight DS-Fi2, Japan). In each sample, four parameters including Johnsen's score, spermatogenesis, miotic index (MI) and seminiferous tubules diameter were evaluated.

Johnsen's score is a semi-quantitative parameter for estimation of sperm production capacity. Therefore, 100 round seminiferous tubules in each sample tissue were selected and scored based on parameters that were listed by Johnsen (1970).

Spermatogenesis was estimated quantitatively in 100 seminiferous tubules. The ratio of tubules with spermatozoa inside them to empty ones was considered as spermatogenesis percentage (Azizollahi et al. 2011). The average diameter of testicular tubules was determined by randomly measuring of ten smallest and roundest tubules per each sample.

For evaluation of cell loss percentage during cell division, meiotic index (ration of round spermatids/primary spermatocytes) was determined in 10 seminiferous tubules and the mean number obtained for each testis.

Statistical analysis

All analyses were performed using SPSS 17 software (SPSS Inc., Chicago, IL, USA). The evaluation of significant difference between the means of experimental groups was performed with using one-way analysis of variance followed by the Tukey test as post hoc. Values were expressed as mean ± S.E.M. (standard error of mean). The significance level was considered $P \leq 0.05$.

Results

Pathologic findings

In the macroscopic inspections of infected testis white mature nematodes were found in the arteries of the spermatic cords. Microscopic examination of infected samples revealed longitudinal and transvers sections of *D. evansi* inside spermatic cords vessels. The lumen of some blood vessels was obstructed by mature parasites. Obliterating endarteritis with predominant eosinophils infiltration was observed that resulted in parasitic occlusion of arterial lumen. The tunica intima of affected vessels showed hyperplasia response. Also, smooth muscles of arterial walls were hypertrophied (Figs. 1 and 2).

In the histopathologic investigation of infected testis, seminiferous tubules were shrinkage, and their basal lamina were thickened and hyalinized. Germinal epithelium of the seminiferous tubules showed degenerative changes in different stages of germ cells including reduced in number and formed vacuolar spaces between them. The lumen of

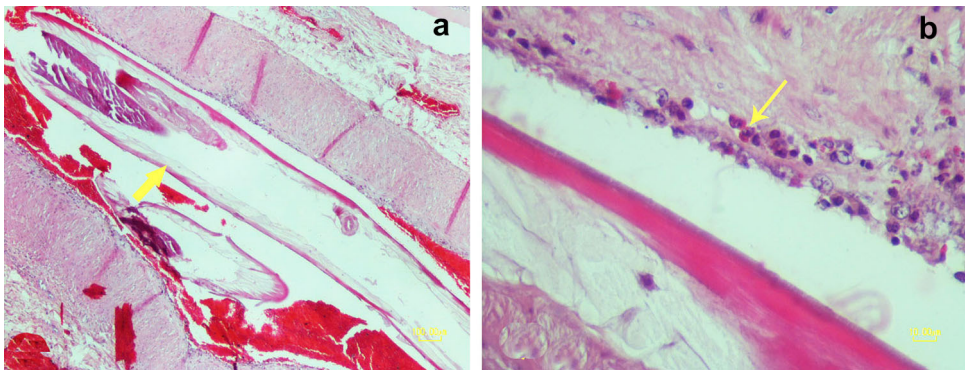

Fig. 1 **a** Longitudinal section of dipetalonema inside the lumen of spermatic cord artery (thick arrow), **b** infiltration of eosinophils in the artery wall (thin arrow)

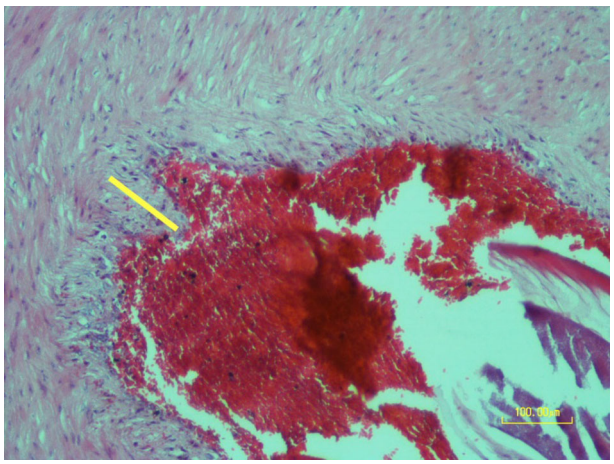

Fig. 2 The adult parasite is present in the lumen of artery (right of the image) that causes occurrence of hyperplasia and thickening of the tunica intima (yellow line) (Color figure online)

some tubules was empty and lack of spermatozoa. Most of the germ cells had necrotic changes and their nuclei were pyknotic. The number and size of Leydig cells were reduced. In the non-infected samples, normal spermatogenesis processes was seen. All stages of spermatogenic cells including spermatogonia, primary and secondary spermatocytes, and round spermatids were present in the most tubules. In most of the seminiferous tubules, spermatozoa were observed in their lumens (Fig. 3).

Morphometrical evaluations of testis

Johnsen's score, meiotic index, spermatogenesis percentage and diameter of the seminal tubules significantly decreased in the infected group in comparison to the non-infected one ($P < 0.05$) (Table 1).

Discussion

In the present study, the five infected samples showed gross lesions and white, slender shape of *D. evansi*. In Iran, Sazmand et al. (2013) reported that 13.89% of camels had mature nematodes in one organ. They observed that the most infections with mature nematodes of *D. evansi* were occurred in the testes and generally macroscopic infection with adult worm significantly was greater in males than females. On the other hand, microfilaria detection in the blood showed no significant difference between male and female camels (Nourollahi Fard et al. 2011). Filariasis by *D. evansi* also can affect other tissues like pulmonary arteries, right auricle, lymph nodes and mesentery (Dakkak and Ouhelli 1987).

Generally, presence of mature nematode in the testicular tissue is a rare condition. Another helminth that could infect testis is *Dirofilaria*. Some studies described attendance of *D. immitis* and *D. repens* in the scrotum, epididymis and spermatic cord (Pampiglione et al. 1999; Theis

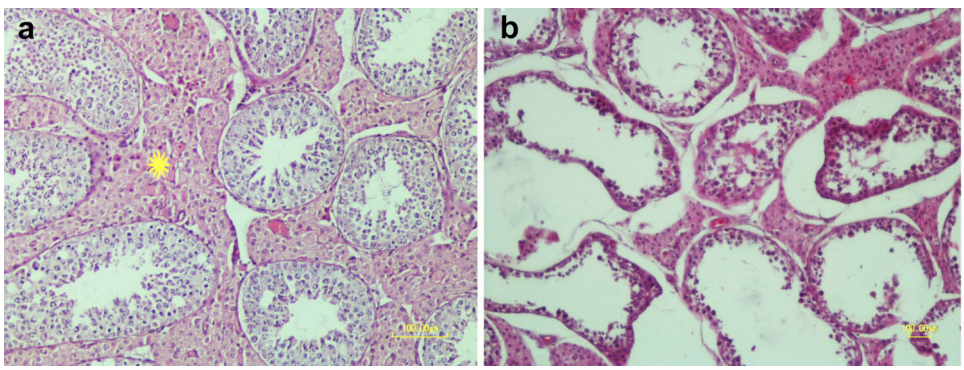

Fig. 3 **a** Normal testicular structure. Active seminiferous tubules with existence of all spermatogenic cell lines. Leydig cells (star) can be seen between the tubules, **b** Destruction of spermatogenic cells. The most seminiferous tubules contain only one row of spermatogonia as well as few numbers of primary spermatocytes

Table 1 The mean ± SEM of seminiferous tubules diameter, Johnsen's score, meiotic index, spermatogenesis percentage in both infected and non-infected testes

Parameter	Infected samples	Non-infected samples	*p*-value
Spermatogenesis (%)	8.60 ± 1.20	50.00 ± 1.54	0.0001
Johnsen score	4.56 ± 0.15	8.06 ± 0.19	0.0001
Meiotic index	1.83 ± 1.39	2.12 ± 1.87	0.011
Seminiferous tubules diameter (μm)	226 ± 2.15	184 ± 1.96	0.0001

et al. 2001; Singh et al. 2010). But, *D. immitis* is primarily pathogen of cardiopulmonary organs and *D. repens* often cause subcutaneous nodules. The reports of infection with *Dirofilaria* were limited to human, cat and dog (Simon et al. 2012). On the other hand, Dirofilariasis of testes is a rare condition and most of its reports belong to subcutaneous form in the scrotum. Whereas, in one study, the prevalence of *D. evansi* in testicular tissue was reported in 50 percent of animals (Mowlavi et al. 1997). So, the filariasis caused by *D. evansi* is a unique parasitic manifestation of mature nematodes in the testicular tissues that is dedicated to the male camels.

In the present study, diameters of seminiferous tubules significantly decreased in the infected samples. This may be due to reduction in the number of germ cells or decrease in the secretory activity of Sertoli cells that led to lowering luminal fluids (Lanning et al. 2002). These changes occurred because of testicular degenerations that affected germ cells division and Sertoli cells function. Degenerative changes were seen in the histopathological evaluation of the infected samples due to the presence of mature nematodes in the blood vessels of spermatic cord. The accumulation of mature nematodes in the spermatic cord arteries can cause ischemia in testicular tissue through the partial or complete obstruction. Additionally, presence of nematodes inside the testicular vessels may disturb the nutrient and gas exchange of seminiferous tubules by physical obstruction and/or inflammation of arterial endothelium. In the present study, arterial walls were thickened everywhere parasites were seen. It is demonstrated that infection with *Elaeophora schneideri* leads to obstruction of carotid and cephalic arteries with adult nematodes and consequent thrombosis and infarction of tissues in the definitive hosts (Adcock and Hibler 1969; Pence 1991). In rare reports, arterial obstruction of spermatic cord with mature nematodes of *Angiostrongylus costaricensis* was associated with necrosis of testicular tissue (Ruiz and Morera 1983; Sánchez-Sierra et al. 2019). Secondly, filariasis of the spermatic cord may interfere with cooling system of testes. There is vital requirement for normal spermatogenesis in mammals that the temperature of testes be 3–6 °C lower than body temperature (Kandeel and Swerdloff 1988). The main mechanism for this testicular cooling system is countercurrent heat exchange between the venous and arterial blood in the spermatic cord. Presence of parasites inside vessels could interfere with this process (Brito et al. 2004). Temperatures upper than normal range in testes can lead to disturbance of spermatogenesis and a loss in testicular weight because of germ cell apoptosis (Shiraishi et al. 2012; Zhang et al. 2012). There is no evidence in the literature that describes temperature inside the testes elevates in resulting of mature nematodes in the spermatic cord or testicular arteries.

At the current study, presence of mature nematodes in the spermatic cord was accompanied with degenerative changes in the seminiferous tubules. Orchitis resulted from *D. evansi* was reported previously (Chhabra and Gupta 2006). Sazmand et al. (2013) demonstrated inflammation, fibrosis and atrophy in testes samples from infected camels with *D. evansi*. They also reported necrosis and sloughing of germ cells, inflammation of vascular wall with hemorrhage in some areas of interstitial tissue and infiltration of eosinophils and lymphocytes. These lesions are similar to what we observed microscopically.

In conclusion, infection with mature nematodes of *D. evansi* can decrease spermatogenesis in testes due to presence of adult nematodes in the spermatic cord vessels.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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