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Full Article

Trichosomoides crassicauda infection in wistar rats

Bahrami[*1], S., Rezaei[2], A., Alborzi, A.R. [1]

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

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ABSTRACT

Laboratory animals, including rats, play an important role in biomedical research and advances. The human care and management of these animals is an ongoing concern. Since, *Trichosomoides* infections in rat colonies can interfere with research protocols it is important to know rate of infection and pathology of the infection in the animals used in experimental studies. 275 rats were eviscerated and urinary bladders were collected. The numbers of collected nematodes from each of the urinary bladders were counted under a stereomicroscope and identified on the basis of morphological criteria. Tissue sections were collected and processed routinely for histopathological studies. Out of 275 urinary bladder of adult laboratory Wistar rats examined, 156 (56.72%) were infected with the nematode, *Trichosomoides crassicauda*. There was significant difference (P<0.05) in infection in female and male rats, with rate of 47.73% and 80.26%, respectively. The number of nematodes collected from each infected rats ranged from one to fourteen with an average of three nematodes per animal. Histopathological evaluation revealed multiple parasites with variable degree of lesions in transitional epithelium of urinary bladder. Parasites were lying upon the epithelium or located in chambers between epithelial cells. Also immature and embryonated eggs were seen in female worms. Other lesions were as follow: Hyperplasia of epithelium, erosions, ulcers and eosinophilic cystitis. This study reports the data on the presence of helminth parasites in laboratory rat colonies, and suggests paying attention on controlling the sanitary conditions of animal houses.

Keywords: Wistar rats, *Trichosomoides crassicauda*

INTRODUCTION

As rodents, such as mice and rats are the most common laboratory animals which used in research and testing, the health surveillance of them is important due to animal ethics and welfare. They are seldom investigated for autochthonous ecto and endoparasites. *Trichosomoides crassicauda* is a hair-like nematode which colonies in the urinary bladder of the laboratory and wild rats. This parasite is member of the order *Enoplida* and is therefore phylogenetically closely related to *Trichuris* and *Trichinella sp.* (Zubaidy and Majeed 1981). For the first time Bellingham (1840) described the nematode as a non-pathogenic worm in rats. The eggs are passed with the urine and ingested by the next host and they hatch in the stomach. Prepatent period of the parasite is 8-10 weeks. Movement of

*Author for correspondence.Email: s.bahrami@scu.ac.ir

larvae from the digestive tract to the lungs, and finally to the urinary organs, takes place through the abdominal, thoracic cavities and the blood circulation. During pregnancy the larvae cannot penetrate the placental barrier (Hasslinger and Schwarzler 1980) but the infection is usually transmitted from mother rats to their offspring before weaning. As *Trichosomoides* infections in rat colonies may interfere with research protocols due to stress induction, it is necessary to record every case available with the view of revealing the prevalence of this parasite. Based on our knowledge there are few published information on the prevalence of *Trichosomoides* in laboratory animals in Iran (Alborzi 2007). This paper is intended to report infection and the pathological changes in the urinary bladder of Wistar rats naturally infected with *Trichosomoides crassicauda* in Khuzestan province of Iran.

MATERIALS AND METHODS

Two hundred seventy five (275) adult laboratory Wistar rats (199 female and 76 male) were randomly selected from both sexes in animal house of faculty of veterinary medicine in Ahvaz, southwestern Iran. Their average weights were 220±20 gram. The animals were housed at a temperature of 24±2°C and relative humidity of 40% to 70% with weekly floor exchange. They had free access to water and standard pelleted laboratory animal diets. A 12:12 light: dark cycle was followed in the mentioned animal house center. During 2010 to 2012 all the animals were randomly collected from those accidentally or humanely killed in practical classes or various research studies (such as zoology or physiology and etc.) in different laboratories of the faculty. Most of the animals were sacrificed humanely by placing them in a glass desiccator jar for open-drop anesthesia with chloroform following standard animal ethics guidelines of Iran. Consequently, necropsy was done on all animals and gender determined by visual inspection of sexual organs. All rats were eviscerated and urinary bladders were collected. The numbers of collected nematodes from each of the urinary bladders were counted under a stereomicroscope and identified on the basis of morphological criteria according to Soulsby (1986). Urinary bladders were fixed in 10% neutral-buffered formalin solution, processed routinely for paraffin embedding, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E).

Statistical analysis. Fischer Exact and Chi-square tests were used to compare infection rates among sexes. P value of <0.05 was considered statistically significant.

RESULTS

Out of 275 urinary bladder of adult laboratory Wistar rats examined, 156 (56.72%) were infected with the nematode, *Trichosomoides crassicauda*. Of the 199 urinary bladders from female rats, 95 (47.73%) and of 76 urinary bladders from males 61 (80.26%) were infected with the nematode. The infection in male rats was significantly higher than the female ones ($p<0.05$) (Table1).

Table 1. The rate of *T. crassicauda* infection in Wistar rats according to the sexes.

Sex	Total	Infected	Non-infected	rate (%)
Male	76	61	15	80.26
Female	199	95	104	47.73
Total	275	156	119	56.72

The minimum and maximum infection rate was 1 and 14, with an average rate of infection of 3 nematodes per animal. Histopathological evaluation revealed multiple parasites associated with lesions in transitional epithelium of urinary bladder. In most of infected lesions parasites were seen lying upon the epithelium or were located in chamber-like structures between transitional epithelium cells. The wall of these chambers was lined by epithelial cells (Figures 1 and 2). Erosions and ulcers due to detachment of epithelium were present (Figure 3). Hyperplasia of epithelium also was obvious and in severe cases it was papillomatous in shape. It was characterized by proliferation of epithelium and increased thickenings of urothelium and at some places the epithelium was more than 5 layers thick around the burrowed worms in the bladder mucosa (Figure 2). Immature eggs and embryonated eggs were seen in female worms (Figures 4 and 5). The bladders with embryonated eggs showed severe lesions.

Eosinophilic cystitis was characterized with infiltration of eosinophils and hyperemia in the lamina properia.

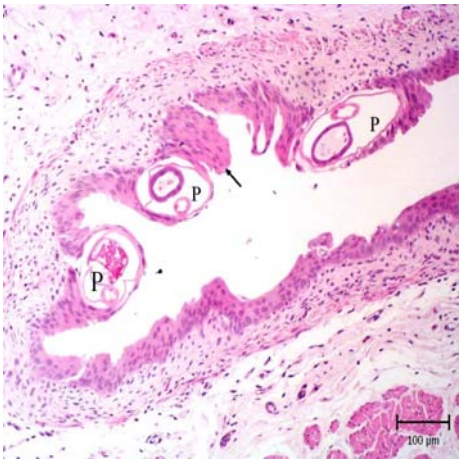

Figure 1. Sections of *T. crassicauda* (P) are located in chamber like structures between epithelial cells. Note to hyper plastic urothelium (Arrow) (H & E, Bar: 100µm).

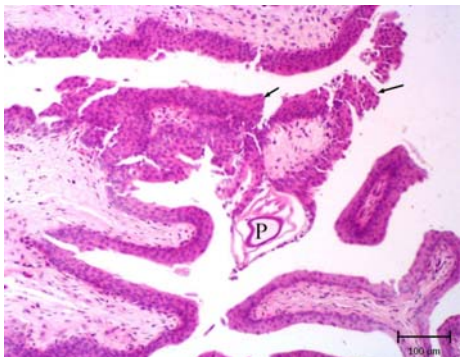

Figure 2. *T. crassica*uda (P) is embedded within urothelium and hyperplastic epithelium (arrows) are obvious (H & E, Bar: 100µm).

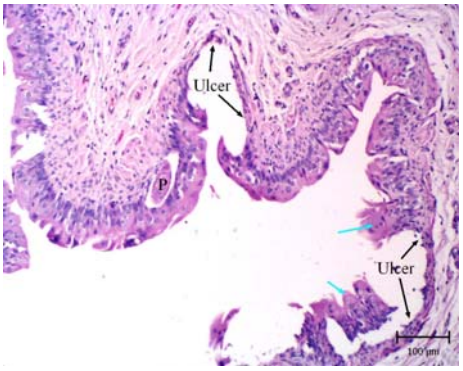

Figure 3. Multiple ulcers are seen in urothelium and also a section of burrowed *T. crassicauda* is obvious. Blue arrows indicate hyperplastic area of epithelium (H&E,Bar: 100µm).

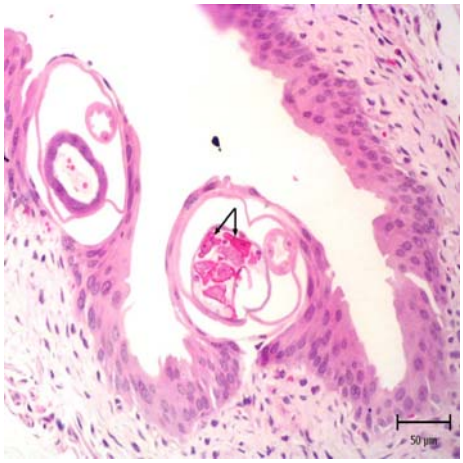

Figure 4. Eggs (arrows) within female worm of *T. crassicauda* with eosinophilic walls are indicated (H&E, Bar: 50µm).

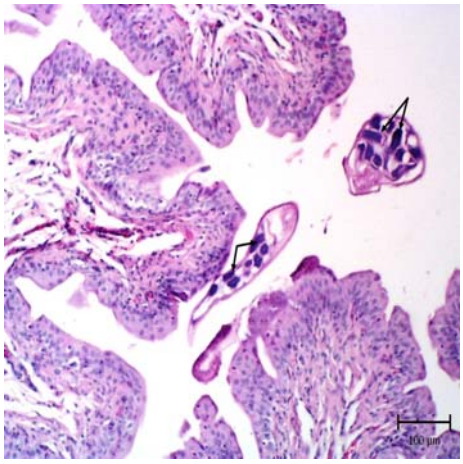

Figure 5. Embryonated eggs (arrows) are visible within female worms of *T. crassicauda* which are located upon the urinary bladder epithelium (H & E. Bar: 100um).

DISCUSSION

Biomedical research still depends on the use of laboratory animal models. Mice and rats are the most common laboratory animals used in research and experiments. Despite the presently intensive use of isolated cells and molecules and *in vitro* methods such as tissue cell culture, recombinant DNA and monoclonal antibodies, biomedical research still depends to a large extent on data obtained from experiments performed with living laboratory animal models. Therefore, sensitivity and accuracy of the results may be compromised when these animal models are infected. As a

consequence, infected animals are not suitable for experimental studies (Vessel *et al* 1976, Melby & Balk 1983). Besides other infectious agents, parasites can complicate research by inducing physiological and immunological alterations in the hosts. A common urinary disease in rat colonies is nematodiasis. This occurs when the nematode parasite *T. crassicauda* infects the rat's urinary bladder, causing painful urination, stunted development, and stones in the bladder. Kidney diseases like pyelitis, renal pelvic inflammation and uroliths may also occur if the parasite moves upward from the bladder. Contamination occurs by ingestion of *T. crassicauda* embryonated eggs, which are expelled through the urine of infected animals. In the present study high rate of infection were detected in rats. The high rate of parasite infection was due to the absence of a quarantine program and effective sanitary barrier systems able to keep animals under controlled sanitary conditions. Thus the high rate of infection can be related to overcrowding. After evaluation of rats cages, it was noticed that the number of animals in the cages were not at a standard level. In this study, the sex of rats had significant effect on the rate of infection, which could be due to the sexual behaviors of male rats. These findings were in accordance with the study by Bone & Harr (1967), but this data is in contrast with the results obtained by Zubaidy and Majeed (1981). In the present study the main feature of the infected bladders was the presence of nematode parasites (lying upon epithelium or located in chambers between epithelial cells) associated with hyperplasia, erosion and ulcers in the urothelium. Cornish *et al* (1988) reported that the female worms were frequently filled with immature eggs, embryonated eggs and often with sections of male worm buried in the stabilized mucus. They showed the changes by scanning electron microscope. In this study the sections of female worms with immature eggs or embryonated eggs which were embedded in the epithelium were seen in chambers with epithelial cells and mucus. The erosion and ulcers may be associated with the existence of female worms from chambers. According to Cornish *et al* (1988), the presence of worms cannot be reliably diagnosed by examination the bladder mucosal surface and through histopatological examination of paraffin wax sections. This is in contrast with the results of current research. In this paper, two inexpensive methods were used for detecting *T. crassicauda* infection. In Serakides *et al* (2001) investigation among the bladders that contained the parasite variable degrees of epithelial hyperplasia, papilloma and inflammation with or without urolithiasis was seen. Antonakopoulos *et al* (1991) reported that the bladder epithelium of *T. crassicauda* infected rats showed a diffuse, mild, flat hyperplasia, four to six cells thick. Ozkorkmaz (2011) showed that in diabetic rats that were infected with *T. crassicauda* the epithelium of the urinary bladder were stratified and cystitis, hyperplasia and epithelial papilloma and inflammation were seen. Hyperplasia and inflammation is in agreement with other research findings and urolithiasis was detected in 10% of the rats examined in this study. In this research eosinophilic cystitis with accumulation of eosinophils in the lamina properia was seen. This finding is in contrast with Tabaran *et al* (2011) who reported granulomatous reaction in the lamina. This lesion may be due to aberrant penetration of worms. Some studies showed that in the infection with Trichosomoides urine becomes bloody and bladder tumors have been seen depending on the severity of the infection. The presence of the nematode has been reported to be associated with the formation of urinary calculi and increase in leucocytes in the transitional epithelium of the urinary tract. In Serakides et al (2001) study persistent eosinophilic infiltration and bladder tumor has also been reported, but a definite causal relationship has not been established. In this study preneoplastic and dysplastic lesions were not seen. The fact that many laboratory rat colonies were found to be infected suggests a need for eradication and

improvement of the quality of laboratory rodents in Iran. The use of laboratory animals bred under barrier_ maintained system, controlled environment conditions and submitted to periodical genetic and sanitary monitoring is of utmost importance.

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