Annals of Parasitology 2018, 64(3), 235–240
doi: 10.17420/ap6403.158

Original papers

A survey of *Dictyocaulus arnfieldi* (Nematoda) infections in equids in Urmia region, Iran

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ABSTRACT. *Dictyocaulus arnfieldi* is a lungworm commonly found in equids; however, relatively little is known about it. The aim of the present study was to establish the prevalence of equine lungworms in naturally-infected working and sporting horses, donkeys and mules in rural areas of Urmia, northwest Iran. The fecal samples were collected from 299 working horses, 57 sporting horses, 66 donkeys and 37 mules during the period March 2014 to June 2016. The collected fecal samples were processed within 48 hours following rectal sampling. The larval count/g (LPG) of feces was determined using the Baermann technique. The larvae were identified morphologically, and the arithmetic mean of the LPG was calculated at each sampling point. The overall prevalence of lungworm in all equine species was found to be 19.20%., with specific values of 15.71%, 0%, 31.81% and 24.32% being found in working horses, sporting horses, donkeys and mules, respectively. The working horses, donkeys and mules were also found to be heavily infected with *Dictyocaulus arnfieldi* and in addition to donkeys and mules, working horses are considered natural hosts for lungworm, and this has effects on both the epidemiology of parasitic infections and performance of the equids.

Key words: *Dictyocaulus arnfieldi*, working horse, mule, donkey

Introduction

Although the equine lungworm, the nematode *Dictyocaulus arnfieldi*, has a worldwide distribution, it is particularly common in temperate areas [1]. *D. arnfieldi* can infect all equid species, with donkeys and mules being considered the natural reservoir hosts [1–3]. Young donkeys may display persistent hepatic infection [4], and hence can represent a predominant source of pasture contamination. However, while patent infections may persist throughout the life of a donkey, they are generally confined to foals in the case of horses [5]. Overt clinical disease associated with *D. arnfieldi* infection is typically not observed in reservoir species, even in the face of prolonged patent infections or very high parasite burden [1,2]. In contrast, such clinical signs of respiratory disease caused by *D. arnfieldi* infection are observed in horses and ponies [6,7]. Coughing and elevated respiratory rate, as well as other clinical signs, may be seen as soon as 12 days after ingestion of third stage larvae, but patency probably does not develop for 12 to 14 weeks [1]. Although clinical disease is rare in donkeys, *D. arnfieldi* infection has been associated with increased susceptibility to respiratory viruses including influenza [8].

D. arnfieldi has a direct life cycle, and a prepatent period of approximately 12 weeks [1]. The endpoint of the parasite cycle emerges when the infective larvae arrive into the lungs from the intestine via the lymphatic system. Final maturation occurs in the bronchi [1]. Mature worms are present in the smaller airways, and they lay eggs which, being already embryonated, can swiftly hatch into first-stage larvae [9]. L1 hatches almost immediately after eggs are passed in the feces and spread on pastures [1].

Infected donkeys pass eggs throughout the year; however, fecal larval production increases through

the spring and summer seasons, and in temperate climates, environmental conditions may favor larval development to the infective L3 stage during the summer [3,10]. *Dictyocaulus arnfieldi* has been recorded in locations all over the world including Iraq, Turkey, Ethiopia, Netherlands and the U.S.A. [4,7,11–15]. There has only been one report in Iran regarding *Dictyocaulus arnfieldi*: the parasite was identified in a horse kept in a zoo in northeast Iran, which demonstrated a chronic non-productive cough [16]. In spite of the importance of horses in North West of Iran, no investigation on the prevalence of *D. arnfieldi* has yet been carried out.

The aim of the present study was to determine the prevalence of equine lungworms in naturally-infected horses (working and sporting), donkeys and mules in rural areas of Urmia.

Materials and Methods

Study region. Fecal specimens were gathered from rural areas around the city of Urmia in the northwest of Iran. The region has a semi-humid climate, with a mean rainfall of 350 mm, maximum monthly temperature of 28.3°C in August and a minimum monthly temperature of -5°C in January. It is bordered by Turkey and Iraq. Due to the arduous nature of the mountain routes, some residents of the area commonly use working horses, donkeys and mules to travel across the borders and carry goods. Sporting horses are kept entirely in racing clubs in single boxes, are fed by their owners and receive regular medical treatment, including anti-parasitic medication every two months. In contrast, working horses, donkeys and mules are kept in groups in barns, and while they are entirely fed in their stalls in autumn and winter, they are grazed in pasture or stall fed during the spring and summer; in addition, they had not received any medical treatment before the study.

Animals. All animals, including working horses, sporting horses, donkeys and mules were clinically examined before sampling. In addition, the following data was collected: sex, age, owner, type of housing, access to pasture, mixing equids with each other, stocking density in pasture, and barn and deworming protocol. Also, their age and gender were recorded (Tables 2,3).

Sample collection. From March 2014 to June 2016, fecal samples were collected from 299 working horses, 57 athletic horses, 66 donkeys and 37 mules. Fecal samples were collected directly from the rectum, usually in the morning, into a labelled container using sterile gloves. If immediate processing was not possible, the samples were kept in a refrigerator at 4°C; however, all samples were processed within 48 hours. The larval count/g of feces (LPG) was determined using the Baermann technique [17]. Standard Baermann apparatus was used, comprising a funnel (glass or plastic, depending on processing method) and a 40-mesh per μm brass wire screen placed approximately 4 cm below the top of the funnel. A rubber tube was connected to the funnel bottom, and a clamp was attached to the tube approximately 10 cm below the funnel. The funnel was filled with warm tap water (23–28°C), and a filter was placed over the wire screen. The larvae were identified morphologically [18], and the arithmetic mean of the LPG was calculated at each sampling point. Samples were considered as positive if at least one larva was observed.

Statistical analysis. Statistical analyses were performed using SigmaSta for Windows version 3.5 (Chicago, IL). The Chi-Square test was used to evaluate the differences in prevalence between different equine species, age groups and sexes. Differences between equine species with regard to LPG were compared using the Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Dunn's method for pairwise comparison.

Table 1. Prevalence of *Dictyocaulus arnfieldi* identified in equids

Species	Total examined	No. of positive (%)	95% Confidence interval
Working horse	299	47 (15.71)[a]	0.12–0.20
Athletic horse	57	0 (0)[b]	0.0.0–0.08
Donkey	66	21 (31.81)[c]	0.21–0.45
Mule	37	9 (24.32)[acd]	0.12–0.40
Overall	459	77 (19.20)	0.15–0.22

Different letters indicate statistically significant differences ($P<0.05$)

Results

The results of present study revealed an overall prevalence of 19.2% equine lungworm (Fig. 1), with the specific prevalence values being 15.71%, 0%, 31.81% and 24.32% in working horses, athletic horse, donkeys and mules, respectively (Table 1). with statistical significance among them (P<0.05). It was also found that age and sex status did not significantly affect the prevalence of infection (Tables 2,3). LPG in donkeys has the highest level and there was a significant difference for LPG (P<0.5) between donkeys and horses (table 4). However, comparison of LPG of donkeys with mules and horses with mules did not show significant differences (P>0.05). Respiratory signs were detected in 23 working horses (eight infected and 15 uninfected) and two infected mules, but it was not observed in any examined athletic horses, donkeys or uninfected mules.

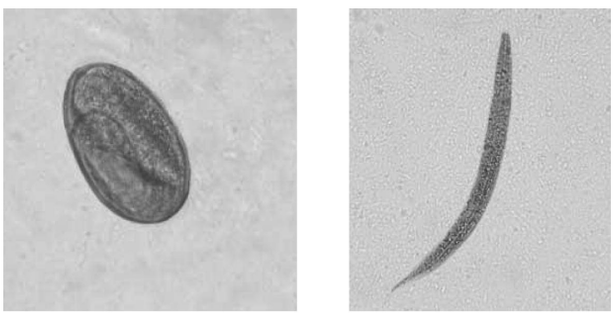

Fig. 1. *Dictyocaulus arnfieldi* larva and egg, 40×

Discussion

The present study compares the infection rate of *D. arnfieldi* among equine species in northwestern Iran. Our findings indicate that infections with *D. arnfieldi* were reported more commonly in donkeys (31.81%) than in horses. The former are considered more common hosts for this parasite, with patent infections sometimes persisting in donkeys throughout their lives. These animals therefore provide the most important sources of pasture contamination [5]. In a study carried out on the lungs of 423 Moroccan donkeys, 47% of the tested animals were found to be positive [19]. A study of *D. arnfieldi* in horses and donkeys from Denmark found that 87.5% of the tested donkeys excreted L1 larvae, usually in high numbers, without any

Table 2. Prevalence of *Dictyocaulus arnfieldi* in different gender of equids

Species	Gender	Total examined	No. positive	Prevalence (%)
Working horse	M	202	34	16.83
	F	97	13	13.4
Athletic horse	M	24	–	–
	F	33	–	–
Donkey	M	66	21	31.8
	F	–	–	–
Mule	M	29	7	24.13
	F	8	2	25

No significant difference was found between genders

Table 3. Prevalence of *Dictyocaulus arnfieldi* in different age groups of equids

Species	Age (year)	Total examined	No. positive	Prevalence (%)
Working horse	<5	49	7	14.28
	5–10	226	39	17.25
	>10	24	1	4.16
Athletic horse	<5	25	–	–
	5–10	18	–	–
	>10	14	–	–
Donkey	<5	7	2	28.57
	5–10	29	9	31.03
	>10	30	10	33.33
Mule	<5	6	1	16.66
	5–10	19	5	26.31
	>10	12	3	25

No significant difference was found between age groups

Table 4. Larva per gram (LPG) of feces of *Dictyocaulus arnfieldi* found in equids

Species	Median (interquartile range)
Working horse	22 (15.25–30)[a]
Mule	55 (40–69)[ab]
Donkey	76 (26–125)[b]

*Different letters indicate statistically significant differences (P<0.05)

considerable clinical signs: interestingly, 106 horses grazed on farms where donkeys were present, and 10.4% of these horses also passed *D. arnfieldi* L1 in their feces [20]. The results of a German study revealed a prevalence of 16.2% in donkeys but no infection in horses [21]. The observed differences between these two studies can be attributed to differences in the numbers of animals examined or in the environmental conditions, which may have affected the development of free-living larvae. In a large-scale study carried out on 5,437 live horses, ponies, mules and donkeys [22], 2% of 5,379 horses were positive for L1, with these infected horses being present on 34 of 90 farms. In comparison, 54% of donkeys and mules had L1 positive samples, and these animals being present on 8 of 10 farms. The present prevalence of lungworm infection in mules was 24.32%. Several studies have found 30% of tested mules to be infected with *D. arnfieldi* [3,7,23].

In the present study, none of the tested sporting horses were found to be positive for *D. arnfieldi.* These horses were kept in equestrian sport clubs with regular anthelmintic programs and there was no history of contact with donkeys and mules. It is possible that these animals were not infected as they had been regularly treated with anti-parasitic drugs before the study. Compared to other infected equids for *D. arnfieldi,* working horses had the lowest infection rate; however, the rate of infection in working horses was higher than that of previous studies [1,6]. These horses were working horses used for heavy physical work and were raised with mules and donkeys. The prevalence of *D. arnfieldi* in horses between the 1980s and 1990s in the United States and Scotland has been reported to range from 2% to 11% [3,7,8]. Although the primary source of *D. arnfieldi* infection for horses is believed to be exposure to larvae that have developed from parasite eggs shed in feces by donkeys [1,6,21], horse-to-horse transmission has been observed in horses with an unknown history of exposure to donkeys or other reservoir hosts [1,6], and although patent infections have also been identified in both adult and young horses and ponies under natural and experimental conditions, these are less common than in donkeys [3,25]. In adult horses, this may be sufficient to perpetuate the life cycle even in the absence of donkeys and foals. Dutch clinical trials on the faeces of 3,791 horses and ponies found a total of 0.2% of the animals to be positive for *D. arnfieldi* L1 [26]. In a separate survey on 300 adult horses admitted to a veterinary clinic specifically for examination of the respiratory tract, respiratory secretion analysis and endoscopy found 2.6% to harbour lungworm infection. In addition, six out of 12 horses that had a history of grazing with donkeys also had lungworm infection [8]. In these studies, considerable variability in prevalence was noted among farms surveyed; this variation was attributed to differences in management or parasite control programs [6]. Recently, only a few works have focused on the global distribution of *D. arnfieldi*; however, it has been suggested that its prevalence has decreased, particularly in areas with effective anthelmintic administration [27].

The present study found 23 of 299 tested working horses and three of 37 mules showed respiratory signs including nasal discharge, coughing, abnormal breath sounds and dyspnea; significantly, none of the infected donkeys or mules showed signs of respiratory tract involvement. However, the observed respiratory signs in horses positive for *D. arnfieldi* could not be exclusively attributed to the parasite infection, and the same could be said for the uninfected horses. Clinical signs associated with *D. arnfieldi* infection can develop as early as 12 days after the ingestion of third stage larvae [7]. Experimental infections resulted in the development of an afebrile condition characterised by coughing, increased respiratory rates and forced expiration. Coughing may persist for several months. Heavy infestations in donkeys do not cause clinical illness [8]. Horse foals may also be symptomless, although some show clinical signs [20].

Dixon et al. [23] reviewed the history and clinical observations of 300 referred equines with respiratory signs. Of these, seven horses were found to be positive for *Dictyocaulus*, all of which suffered from coughing, four of which lived permanently outdoors, three of which had a history of recent pasture change and six had a history of grazing with donkeys. In an experimental study on 49 ponies, 24 showed overt disease but patent

infections were detected in only two [1].

It has been recommended that when establishing a diagnosis in a horse with overt respiratory signs, a history of commingling with donkeys and/or grazing in common pastures should be taken into consideration [8]. However, not all horses co-grazed with infected donkeys show clinical signs of respiratory disease [5]. In fact, horses seem to vary in their response to the parasite; for instance one mare which passed 1,520 L1/g of feces and harboured 20,000 adult worms at necropsy had no clinical signs of lungworm disease [1].

In agreement with other studies [1,8], we did not observe any differences in the infection rates between sexes. In addition, no significant difference was observed between age groups, probably due to the small number of samples from younger horses. The horses of the rural areas of Urmia are mainly raised in the border area and used for transportation. The owners do not use these horses for breeding, and adult horses are supplied from other regions. Horses of all ages are susceptible to parasitic pneumonitis but adults age five to eight years are particularly vulnerable [6]. The LPG levels have been found to be higher in donkeys (76) than in working horses (22) or mules (55). In a report of *D. arnfieldi* infection in a horse in Mashhad, Iran LPG was 5 [16]. A study examining the efficacy of Eprinomectin pour-on against naturally-occurring *D. arnfieldi* infection in donkeys found the arithmetic mean of the LPG count to range from 33.8 to 44.8 in the control groups [28]. An epidemiological study in Morocco on 423 donkeys found the mean number of worms per animal to be 39.5 and the average ratio of worms in lungs to LPG in feces ratio was 1:0.97 [19]. Our findings show that although donkeys and mules had a higher prevalence and larval excretion rate of *Dictyocaulus arnfieldi* than horses, working horses still play an important role in the shedding of eggs and pasture contamination.

The results of the present study showed that the working horses, donkeys and mules were moderately infected with *D. arnfieldi*; however, the sporting horses kept in an equestrian sports club were free from infection. It was also found that in addition to the donkeys and mules, patent infection occurs clearly in working horses. The working equids of the region are used for transportation in mountainous areas and are raised in inappropriate conditions. These animals suffer from malnutrition, especially in cold seasons, and do not receive any regular strategic or tactical anthelmintic treatments, and the epidemiology of parasitic infections and performance of the horses of the region appears to be influenced by these conditions.

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Received 07 January 2018
Accepted 29 May 2018