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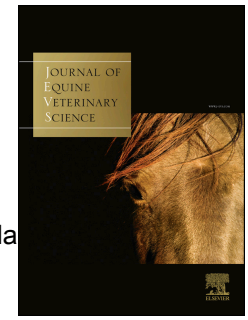

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Prevalence of *Giardia* assemblages among equines in Jordan

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Abstract

A cross-sectional study was carried out on 400 equines holdings (326 horses and 74 donkeys) samples to determine the prevalence of *Giardia* assemblages A, B and E in Jordan. Identifying the *Giardia* assemblages was carried out using ELISA as a screening test and PCR-RFLP targeting Beta giardin loci. In addition, PCR targeting triose phosphate isomerase gene (*tpi*) specific for assemblage A and B were used as confirmatory. 34 samples tested positive by ELISA for *Giardia* with an apparent prevalence of 8.5%. The PCR-RFLP test confirmed *Giardia* assemblages in 30 of the 34 ELISA-positive samples giving a true prevalence of 7.7% (95% CI; 4.8-10.1). Of the 30 positive animals/holdings, 18, 4 and 8 had assemblage A, B and E. Assemblage A was significantly ($p < 0.05$) more prevalent when compared to assemblages B and E. The total infection rates of *Giardia*, assemblage B and E were significantly ($p < 0.05$, Chi-square) higher in donkeys 14.8%, 2.7%, 5.5% compared to horses 5.8%, 0.6%, 1.2%, respectively. Analysis of risk factors revealed that only season was significantly associated with the different *Giardia* assemblages. Autumn (OR = 0.09) was associated with *Giardia* infection regardless of the assemblage type as reducing factor. The odds of infection of assemblage A and E increased in winter (OR = 6.8) and spring (OR = 4.5), respectively. *Giardia* assemblages A, B, and E infect both horses and donkeys in Jordan with potential impact on human and animal health and the odds of infections is significantly associated with season.

Keywords: Giardia assemblages; donkey; horse; prevalence; risk factors; climate; season; Jordan

1. Introduction

Giardia duodenalis (also known as *G. lamblia* and *G. intestinalis*) is very common intestinal pathogen of humans and domestic animals worldwide in both temperate and tropical climate zones [1]. Acute *Giardia* infection is characterized by diarrhea in both humans and animals, while chronic infection in children and young animals are reportedly associated with failure to thrive, wasting and malabsorption syndrome. *Giardia* is usually transmitted by the fecal-oral route, both directly and indirectly [2, 3]. The *G. duodenalis* is the only known species that infects human and most mammals [4]. It is considered as species complex, which has eight assemblages (A to H) identified by sequencing the ribosomal RNA gene and have been supported by sequencing other genes [3].

Assemblages A and B were most commonly isolated from humans and presumed zoonotic [5]. In addition, there is more variation with assemblages where it can be divided into sub-assemblages as AI, AII, and AIII, according to glutamate dehydrogenase (*gdh*) gene sequences [4].

Compared to other animals, only few studies have been conducted to investigate *Giardia* infecting equine, mainly horses. Infection rate varied between 1.5 % in China to 71% in the USA [6-12]. On the other hand, there is only one report about *Giardia* infection among donkeys in China [13]. In Jordan, although giardiasis was included in WHO's neglected diseases initiative [14], *Giardia* infection was never evaluated in animals, and there are only few reports on *Giardia* prevalence in human [15-19]. Recently, two assemblages of *Giardia* were isolated from 48 Jordanian human patients where assemblage A was found in 44.9% of samples while assemblage B was found in 57.1% of samples [20].

The main objective of the study reported here is to investigate the prevalence of *Giardia* assemblages A, B and E infections in horses and donkeys. Furthermore, potential risk factors associated with *Giardia* infection were explored.

2. Methods

2.1 Study area and population

Four out of the 12 Jordanian Governorates located in four climate zones were included in the study: Irbid, Mafraq, the Jordan River Valley and Amman. The Irbid area is located at the north-western parts of Jordan characterized by warm temperate climate mostly planes and semi-hilly in nature. Equines are mainly used in the studied areas for transportation and ploughing lands. The Jordan River Valley is the natural western border of Jordan and situated 200 to 400 meters below sea level and is characterized by having a warm steppe climate. On the other hand, the Mafraq area is located at the eastern part of Jordan characterized by cool desert climate. The Amman area is located at the middle part of Jordan characterized by cool rainy climate and is considered mountainous in nature and is located at about 1000 meters above sea level. Equines are mainly used in the studied areas for transportation and ploughing. As the capital of Jordan, equines are mostly used in Amman for sports and horseback riding.

The average seasonal rainfalls were reported to be 775.5, 386.6, 144.7 and 0.0 mm during winter, autumn, spring and summer seasons respectively (Source: Jordan Metrological Department).

The grazing season starts in January and finishes in March in the river valley (warm steppe climate), while in the other areas, it is usually between February through May on green grazing and from June to august in the aftermath of wheat and barley and other crops.

Equines in Jordan are individually owned, with the exception of few horse stables kept for riding and sport activities. In underdeveloped areas, they are used for farming and transportation. While in desert areas, mainly donkeys are used for transhumance sheep and goat farming. Horses are used by tourists for horseback riding and exploring attraction sites such as Petra and Dead Sea. Only horses kept in big stables are vaccinated against tetanus, herpesvirus and influenza.

2.2 Sample size determination

The prevalence of *Giardia* among horses in Jordan or the Middle East has not been previously reported. However, as there is no previous work on the prevalence of *Giardia* assemblages' among equines in Jordan or the Middle East, prevalence rate of 50% was assumed. According to Thrusfield [21] for an expected prevalence of 50%, the approximate number of samples to be examined is 384 at 95% level of confidence and 5% absolute precision. Representative samples were selected according to estimated density in each study area. A total of 400 samples were collected. The study had at least 80% power at the 5% significance level to detect an odds ratio (OR) ≥ 2 for risk factors present in 50% of controls, and an OR ≥ 3 for those present in 20% of controls.

2.3 Samples and sampling

In the period from September 2014 to August 2015, a total of 400 systematic fecal samples were collected using a list of equine holdings in each of the included areas. A total of 400 households were systematically selected as every 5th on the holdings list was sampled. Most of the holdings (376, 94%) had one animal only while 22 (5.5%) holdings had 2-5 animals and two stables of 30 and 70 horses. One animal from holdings with 1-5 animals and 10-20% of animals from holdings with more than 5 heads were randomly selected and sampled from each holding. One hundred holdings were sampled during each season employing 25 holdings from each climate zone. Thus, 400 different equine households in the study areas were employed (n= 326 horses and n= 74 donkeys). The age of enrolled horses ranged from 2 months to 22 years, with 218 females and 182 males. The age of enrolled donkeys ranged from 1 to 9 years, with 22 females and 52 males. Sampled animals appeared healthy with no clinical sign of diarrhea or weight loss.

At least 5 grams of fecal samples were collected directly from the rectum using clean disposable gloves. The collected sample was mixed well and aliquots were preserved by transferring 0.5 grams of the fecal sample to 2 ml tubes contains 1 ml 2.5% potassium dichromate and stored at 4°C until being tested. At the time of sample collection, five variables including; the time of sample collection, equine species, animal age, gender and location were recorded and entered in MS Excel file.

2.4 The Enzyme-Linked Immunosorbent Assay (ELISA)

All fecal sample were screened for *Giardia* using commercial ELISA kit, (RIDASCREEN®, R-Biopharm, Germany) detecting *Giardia*-specific antigen 65

(GSA 65) according to manufacturer's instructions. The color change was detected using the ELISA reader (Dynatech MR5000, Guernsey, UK) at a wave length of 450 and 630. The samples were considered positive if the reading exceeded the negative control by 0.150 OD according to manufacturer's instruction. The test was reported with 96 % sensitivity and 100% specificity according to the manufacturer's instructions.

2.5 DNA extraction

All ELISA positive *Giardia* samples were subjected to DNA extraction using QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to manufacturer instructions with modification. Before extraction, samples were subjected to 5 cycles of freezing under liquid nitrogen and thawing at 95ºC to break down the cyst walls. Extracted DNA was stored at -20°C for further PCR analysis [22].

2.6 PCR amplification and RFLP analysis

DNA molecular analysis was performed using nested PCR for the amplification of targeted region within the β-giardin gene. In the first PCR reaction primer pair; forward G7 (5′-AAGCCCGACGACCTCACCCGCAGTGC-3′) and the reverse primer G759 (5′-GAGGCCGCCCTGGATCTTCGAGACGAC-3′) were used to amplify a 753bp fragment [23]. The second PCR reaction amplifying 511bp fragment was performed using internal primers pair; forward G5 (5′-GAACGAACGAGATCGAGGTCCG-3′) and the reverse G5 (5′-CTCGACGAGCTT CGTGTT-3′) [24]. PCR cocktail for both reactions

consisted of 10µM of each primer, 12.5µl of MasterMix (Promega, Germany), 2µg BSA and 4µl of DNA templet in a total volume of 25µl. PCR was carried out in the thermocycler (Gene Pro Thermal Cycler, model TC-E-96G, China) under the following conditions: initial denaturation for 5 min at 96°C, 35 cycles (20 sec at 95°C, 30 sec at 53°C and 45 sec at 72°C) followed by a final extension of 7 min at 72°C. The second PCR cycling condition was like the first PCR run except the extension time was for 50 sec and final extension time was for 10 min.

The amplified products were digested using 10 U/µl of HaeIII (New England BioLabs, R0108L, UK) in a final volume of 20µl for 3 h at 37°C. Digested pattern visualized on 3% agarose gel with ethidium bromide stain used for assemblage analysis, according to previous reports [24]. Appropriate negative and positive controls for each assemblage were employed. The test has a high sensitivity and specificity comparable to ssrRNA giardia gene [23]. To confirm the RFLP findings another PCR assay using assemblage A and B specific primers targeting the triose phosphate isomerase (*tpi*) gene was performed as described by Bertrand et al. [22].

2.7 Statistical analysis

Data were stored in a database and analyzed using Epi-Info 7 (CDC, Atlanta, Georgia) and SPSS version16.0 [25]. The true prevalence was calculated using apparent prevalence, test sensitivity and specificity according to Rogan and Gladen [26] . The 95 % confidence interval was calculated for the prevalence. Chi-square analysis was used to test the association among the proportions, and the odds ratios were calculated. The dependent variables were: *Giardia* infection regardless of the assemblage type, *Giardia* assemblage A or B or E infections

status, coded as 0 (negative) or 1 (positive) for each. A total of five variables (categories) were tested: species (donkey and horse), gender (male and female), age groups (less than and older than 5 years), climate (cool temperate rainy, cool desert, warm steppe, and warm temperate rainy), and season (spring, summer, autumn, and winter). The screening for the significant variables to be used in the final logistic regression was conducted using Chi-squared test. The variables that were statistically significant $p < 0.2$ (two sided) were included in the multivariable model forward selection. *P* value of < 0.05 was considered significant.

3. Results

Table 1 summarizes the true prevalence results. Of the 400 samples screened by ELISA test, 34 were positive for *Giardia* infection giving an apparent prevalence of 8.5% (95% CI; 5.8-11.2). The PCR-RFLP test confirmed *Giardia* assemblages in 30 of the 34 ELISA positive samples giving a true prevalence of 7.7% (95% CI; 4.8-10.1). Of the 30 positive animals, 18 had assemblage A (63% of infected equines and 68.4% of infected horses), 4 had assemblage B (13.3% of infected equines and 10.5% of infected horses) and 8 had assemblage E (23.3% of infected equines and 21.1% of infected horses). PCR targeting *tpi* gene for assemblage A or B confirmed the results for RFLP.

Assemblage A was significantly more prevalent in equines compared to assemblages B and E (X^2= 10.7, 2 d.f., P = 0.005). There was no significant difference between the prevalence of assemblages B and E (X^2= 1.35, 1 d.f., P = 0.24). There was no mixed infection with *Giardia* assemblages. The infection rates of *Giardia* regardless of assemblage type, assemblage B and E were

significantly ($p < 0.05$, Chi-square) higher in donkeys 14.8%, 2.7% and 5.5% compared to horses 5.8%, 0.6% and1.2%, respectively.

The Chi-square results for the association of *Giardia* assemblages and analysis of evaluated risk factors are summarized in Table 2a, b and c. Only species and season were associated ($p < 0.2$) with *Giardia* infection regardless of the assemblage type in equines (Table 2a). After forward selection, only the season autumn was significantly associated with *Giardia* infection (Table 3). Similarly, species and season were associated ($p < 0.2$) with *Giardia* assemblage E infection (Table 2c) and after forward selection the model revealed that only spring season was associated (Table 3). Of the five variables/categories, age group and season were associated ($p < 0.2$) with *Giardia* infection assemblage A in equines (Table 2b) and after forward selection the model had only winter season (Table 3). With regard to *Giardia* assemblage B infection, species was the only variable associated ($p<0.1$) with this assemblage in the univariable analyses only.

4. Discussion

To the authors' knowledge, this is the first report evaluating *Giardia* prevalence and risk factors among horses and donkeys in different geographical and climate zones in Jordan. The results showed *Giardia* true prevalence rate among equines in Jordan to be 7.7% using ELISA and PCR-RFLP tests. Horses and donkeys living in the included locations were found to be infected with *Giardia* assemblages A, B and E. Also, the findings of the research reported here demonstrated significantly higher true prevalence of *Giardia* assemblages A (4.6%) when compared to assemblages B and E. Donkeys had higher infection

rate than horses, 14.8% and 5.8% respectively. This is considered the first report of the infection in donkeys with *Giardia* assemblages A and E worldwide, while the only publication on donkeys and *Giardia* [13] they do found only assemblage B. Seasons were associated with the infection with high odds during winter and spring and low odds during autumn depending on the type of *Giardia* assemblage.

Table 4a summarizes the previous reports on *Giardia* infection, regardless of assemblages and occurrence among equines worldwide. Previous reports on equines were all in horses except one [13] which included countries of the Middle East, Europe, and North and South America. Infection rates varied between 0 % in California and Nevada in the United States to 19.6% in Iraq employing different diagnostic tests. Most studies used the microscopy test (n = 8) or direct immune fluorescent antibody test (3). The current work showed a lower true prevalence among horses (6.0%) in Jordan using ELISA and PCR tests than most reported rates. The tests used had a high sensitivity and specificity using one sample [23, 27]. In addition, the nature of horse husbandry with restricted movement, limited grazing and low density, might have played a role in lowering the infection rate compared to other parts of the world.

In Jordan, donkeys are exposed to humans, dogs, cats and wild life either directly or indirectly. During the grazing season, donkeys are an integral component of small ruminant (sheep and goats) flocks providing means of transportation for the shepherd and carrying their tools. Also, in some flocks, donkeys are equipped with a bell around its neck and used as a small ruminant flock leader followed by the whole flock in travelling, replacing the head ram. Thus donkeys, especially during the grazing season (spring and summer) are

relatively more exposed to environmental contamination with *Giardia* from infected sheep, goats, dogs and cats. On the other hand, movement of horses is commonly more restricted as they are kept in stables where they are exposed to dogs, cats and wild animals in addition to humans but less so with sheep and goats in contrast with donkeys.

Reports evaluating *Giardia* assemblages in equines are available from six countries in Europe, one country in South America and China (Table 4b). Four studies reported the occurrence of assemblage A in six European countries, Colombia and China. The contribution of both Assemblages A and B to infection ranged from 0 to 50 % while that of assemblage E ranged from 0 to 100%. The current results demonstrated higher proportions of assemblage A in infected horses (68.4%) and donkeys (46%). The prevalence rate in the paper presented here is considered high when compared with the prevalence rate of *Giardia* in horses worldwide (Table 4b).

Other studies reported that B as the dominant assemblage in horses [7, 12, 28]. The variability in the dominant assemblage infecting equines in different countries/areas, especially the presumed zoonotic variants (A and B), might be affected by environmental factors, season and management factors including the contamination of the pasture and/or water resources with human and animal waste. Zhang et al. [13] study on donkeys in China identified 4, 1 and 3 subtypes of *Giardia* assemblage B only.

Most studies reported the occurrence of assemblage E in horses (Table 4b). The percent of assemblage E among evaluated samples ranged from 0% to 100%. In Jordan, among infected samples, 21% of the horses and 36% of the

donkeys had assemblage E with percentages ranked second after assemblage A and ahead of assemblage B and similarly the prevalence (Table 1). Assemblage E has been reported as the dominant assemblage in cattle and sheep [29-31]. Thus, in Jordan, assemblage E is expected to occur more in equines especially donkeys where most (98%) of domestic animal populations are sheep and goats which have a close association with donkeys.

Although this study had the limitation of being conducted during one year period, seasonal influence on the *Giardia* infection rate, regardless of assemblages, was clear in this paper. The prevalence was highest in the winter (13%) and spring (10%) compared to the summer (6%) and autumn (1%). In Jordan, spring and summer are the main grazing seasons. The increase in the odds of infection in winter can be explained by the contamination of the barns, water and the pasture with infective *Giardia* cysts from infected animals. This is exacerbated by overcrowded conditions and the rain water flushing the cysts into the environment, spreading them over a wider area exposing the infection to other animals.

In the United Kingdom, it is believed that transmission of *Giardia* is increased by heavy rain [32]. In Jordan, the winter, autumn and spring seasons are wet while the summer is dry with reported shortages of water. Although the effect of rain falls in autumn on cyst dispersal is minimal compared to winter and spring. This may be explained by soil aridity caused by the long hot dry summers which kill the infective cysts and dries out the soil to a significant depth facilitating the absorption of the precipitation occurring during the autumn season washing down the cysts from the topsoil without significant runoff. Thus *Giardia* contamination in the environment is reduced.

The prevalence of assemblage A is significantly higher in winter (6.8%) while assemblage E was highest in the spring, this can be explained by the source of the infection. As assemblage A presumed to be zoonotic with a winter peak concomitant with the rainy season and the highest rainfall in Jordan. This may be due to the contamination of water resources with the human and animal waste. Conversely, assemblage E which is not considered as zoonotic is more common in hoofed farm animals [29-31]. The peak prevalence of this assemblage is in the grazing season (spring) and may indicate the role of grazing animals in contaminating the environment. Also, giardiasis is more common in young animals as age resistance usually occurs over time. Spring is the lambing and kidding season with the largest population of susceptible young lambs and kids which would potentially shed large numbers of *Giardia* cysts leading to the consequent high environmental contamination in the spring.

Our study was conducted on apparently healthy adult animals which resulted in the limited effect of age on the prevalence. On the other hand, it is important to study these subclinical animals which serve as reservoir for *Giardia* that infect humans and other animals. In addition, although samples were collected from 4 different areas representing variable climate zones, there was only a limited effect on *Giardia* prevalence. The relative proximity between these areas (with 70 km radius), the free movement of animals and trading, and transhumance type of grazing adopted in the Middle East could all explain the minimal area effect on the prevalence.

Although using β-giardin PCR-RFLP is a sensitive technique in detecting *Giardia* assemblages, more work is needed to detect sub-assemblages to confirm the zoonotic nature of the assemblages found compared to the sub-assemblages

found infecting humans in Jordan. In addition, the absence of mixed assemblages infection in our study might need confirmation using other gene target. Finally, wider studies including other animal species and drinking water quality are needed to document the transmission dynamic of *Giardia* in Jordan.

5. Conclusions

The current study documented for the first time the prevalence of *Giardia* including assemblages A, B and E in equine in Jordan and assemblages A and E in donkeys worldwide. The dominance of the presumed zoonotic assemblage A in this study may indicate the role of equines as a source of environmental contamination with *Giardia* with all impact on human and animal health in Jordan.

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Table 1

True prevalence of *Giardia* assemblages detected in equines (n=400) using the ELISA test and confirmed by PCR-RFLP analysis targeting Beta giardin loci and digested with HaeIII enzyme during September 2014 to August 2015.

Subtype	No. +ve	True prevalence (95% CI)
A	18	4.6* (2.7-7.1)
B	4	1.0 (0.02-2.0)
E	8	2.1 (0.8-3.9)
Total	30	7.7 (4.9-10.1)

*Significant Chi-squar test = 10.7, 2 d.f., $P = 0.005$

Table 2a

Univariate association between potential risk factors and *Giardia* infection positivity regardless of the assemblage type, by ELISA and PCR-RFLP tests among 400 equines during the period September 2014 to August 2015 in Jordan

Variable	Category	Coding	Total No.	*+ve* % (n = 30)	*P*
Species	Horses	0	326	5.8 (19)	0.01
	Donkeys	1	074	14.8 (11)	
Gender	Female	0	218	6.4 (14)	0.29
	Male	1	182	8.7 (16)	
Age group (Years)	Up to 5	0	229	8.3 (19)	0.47
	>5	1	171	6.4 (11)	
Climate	Cool temperate rainy	1	100	7.0 (7)	0.39
	Cool desert	2	100	9.0 (9)	
	Warm steppe	3	100	10 (10)	
	Warm temperate rainy	4	100	04 (4)	
Season	Spring	1	100	10.0 (10)	0.01
	Summer	2	100	06.0 (6)	
	Autumn	3	100	01.0 (1)	
	Winter	4	100	13.0 (13)	

Table 2b

Univariate association between potential risk factors and *Giardia* Assemblage A infection positivity by ELISA and PCR-RFLP tests among 400 equines during the period September 2014 to August 2015 in Jordan

Variable	Category	Coding	Total No.	*+ve* % (n = 18)	*P*
Species	Horses	0	326	4.0 (13)	0.30
	Donkeys	1	074	6.9 (5)	
Gender	Female	0	218	4.1 (9)	0.88
	Male	1	182	4.4 (8)	
Age group (Years)	Up to 5	0	229	5.7 (13)	0.19
	>5	1	171	2.9 (5)	
Climate	Cool temperate rainy	1	100	5.0 (5)	0.77
	Cool desert	2	100	6.0 (6)	
	Warm steppe	3	100	4.0 (4)	
	Warm temperate rainy	4	100	3.0 (3)	
Season	Spring	1	100	3.0 (3)	0.01
	Summer	2	100	3.0 (3)	
	Autumn	3	100	0.0 (0)	
	Winter	4	100	12.0 (12)	

Table 2c

Univariate association between potential risk factors and *Giardia* Assemblage E infection positivity by ELISA and PCR-RFLP tests among 400 equines during the period September 2014 to August 2015 in Jordan

Variable	Category	Coding	Total No.	*+ve* % (n = 8)	*P*
Species	Horses	0	326	1.2 (4)	0.02
	Donkeys	1	074	5.5 (4)	
Gender	Female	0	218	1.4 (3)	0.32
	Male	1	182	2.7 (5)	
Age group (Years)	Up to 5	0	229	1.7 (4)	0.67
	>5	1	171	2.3 (4)	
Climate	Cool temperate rainy	1	100	1.0 (1)	0.38
	Cool desert	2	100	2.0 (2)	
	Warm steppe	3	100	4.0 (4)	
	Warm temperate rainy	4	100	1.0 (1)	
Season	Spring	1	100	5.0 (5)	0.03
	Summer	2	100	3.0 (3)	
	Autumn	3	100	0.0 (0)	
	Winter	4	100	0.0 (0)	

Table 3

Multivariable logistic regression models of factors associated with *Giardia* Assemblages A, B and E infection detected by ELISA and PCR-RFLP tests among 400 equines (horses and donkeys) during the period September 2014 to August 2015 in Jordan

Giardia Assemblage	Variable/ Category	Odds Ratio	95% CI	*P*
A, B or E	Season /Autumn[a]	0.09	0.01-0.70	0.01
A	Season / Winter[b]	6.8	2.5-18.6	0.01
E	Season / Spring[c]	4.5	1.2-19.6	0.04

[a]Likelihood ratio of chi-squared (LRX^2): 106.6 on one degree of freedom (d.f.), [b]LRX^2: 131.6 on one df, [c]LRX^2: 69.9 on one d.f.

Table 4a

Previous work on *Giardia* infection regardless of assemblages' type among horses worldwide and donkeys from Jordan only

Country	No. Examined	Infection %	Test used	Reference
Middle East				
Iraq	107	19.63	Microscopy	[33]
Jordan	326	5.8	ELISA & PCR	Current results
	74 donkeys	14.8		
Europe				
Czech Republic	360	5	Microscopy	[34]
Germany	37 foals	5.4	Microscopy	[35]
North America				
USA, California	91	0-3.2	Microscopy	[36]
USA, California and Nevada	58	0	Microscopy	[37]
USA, Colorado	300	0.66 (2)	IFA	[38]
USA, Nevada	305	4.6	IFA	[39]
USA, Ohio and Kentucky	222	13	DIFA	[11]
USA, Florida	223	1.29	Microscopy	[40]
Canada	35	20	IFA	[41]
South America				
Brazil	64	0	Microscopy	[42]
Brazil	396	0.5	Microscopy	[43]

Table 4b

Previous work on *Giardia* assemblages' infections among horses worldwide and donkeys (from Jordan only)

Country	No. Examined	Infection %	Test	Gene	Assemblage % A	B	E	Ref.
Middle East								
Jordan	326	5.8	ELISA PCR-RFLP	β giardin *tpi*	68	11	21	Current results
	74 donkeys	15	ELISA PCR-RFLP	β giardin *tpi*	46	18	36	
Europe								
Italy	150 (120 foals & 30 adults)	13.3	DIFA, PCR	SSU-rRNA	0	0	100	[10]
Italy	431	8.6	PCR	SSU-rRNA, β giardin	43.2	29.7	27	[9]
Belgium,	134 foals	14.2	DIFA, PCR	β giardin *tpi*	45 Mixed A & B (8)	45	10	[7]
Netherland	44 foals	11.4						
Germany	30 foals	10.0						
Greece	190 foals	11.6						
Poland	10	10	DIFA PCR	β giardin	0	0	100	[8]
N. America	8	NA	PCR	SSU-rRNA, β giardin, *tpi*				[28]
Australia	2	NA						
S. America								
Colombia	195	17.4	PCR	SSU-rRNA, β giardin, *gdh tpi*	5.9	94.1	0	[12]
China	262	1.5	PCR	SSU-rRNA	50	50	0	[6]
	181donkeys	15.5	PCR	*tpi* , *gdh*, β giardin	0	100 (4, 1 & 3 subtypes)	0	[13]

High lights:

- This is the first prevalence and genotyping study to document *Giardia* infecting equine in Jordan.
- Prevalence of *Giardia* was more in donkeys when compared with horses
- The most prevalent assemblage in horse was A when compared with assemblage B and E
- Findings of this study suggest seasonal association with prevalent assemblages of *Giardia* affecting horses and donkeys