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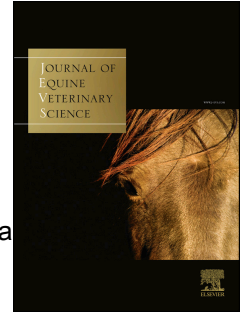
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# Accepted Manuscript

Prevalence of *Giardia* assemblages among equines in Jordan

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**Prevalence of *Giardia* assemblages among equines in Jordan** 1

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**Abstract** 22

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.	95 96 97 98
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.	99 100 101 102 103 104 105 106
<b>2.2 Sample size determination</b>	107
The prevalence of <i>Giardia</i> among horses in Jordan or the Middle East has not been previously reported. However, as there is no previous work on the prevalence of <i>Giardia</i> 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) $\geq 2$ for risk factors present in 50% of controls, and an OR $\geq 3$ for those present in 20% of controls.	108 109 110 111 112 113 114 115 116 117 118
<b>2.3 Samples and sampling</b>	119

In the period from September 2014 to August 2015, a total of 400 120  
systematic fecal samples were collected using a list of equine holdings in each of 121  
the included areas. A total of 400 households were systematically selected as 122  
every 5th on the holdings list was sampled. Most of the holdings (376, 94%) had 123  
one animal only while 22 (5.5%) holdings had 2-5 animals and two stables of 30 124  
and 70 horses. One animal from holdings with 1-5 animals and 10-20% of 125  
animals from holdings with more than 5 heads were randomly selected and 126  
sampled from each holding. One hundred holdings were sampled during each 127  
season employing 25 holdings from each climate zone. Thus, 400 different 128  
equine households in the study areas were employed (n= 326 horses and n= 74 129  
donkeys). The age of enrolled horses ranged from 2 months to 22 years, with 218 130  
females and 182 males. The age of enrolled donkeys ranged from 1 to 9 years, 131  
with 22 females and 52 males. Sampled animals appeared healthy with no 132  
clinical sign of diarrhea or weight loss. 133

At least 5 grams of fecal samples were collected directly from the rectum 134  
using clean disposable gloves. The collected sample was mixed well and 135  
aliquots were preserved by transferring 0.5 grams of the fecal sample to 2 ml 136  
tubes contains 1 ml 2.5% potassium dichromate and stored at 4°C until being 137  
tested. At the time of sample collection, five variables including; the time of 138  
sample collection, equine species, animal age, gender and location were recorded 139  
and entered in MS Excel file. 140

#### 2.4 The Enzyme-Linked Immunosorbent Assay (ELISA) 142

All fecal sample were screened for *Giardia* using commercial ELISA kit, 143  
(RIDASCREEN®, R-Biopharm, Germany) detecting *Giardia*-specific antigen 65 144



(GSA 65) according to manufacturer's instructions. The color change was 145  
detected using the ELISA reader (Dynatech MR5000, Guernsey, UK) at a wave 146  
length of 450 and 630. The samples were considered positive if the reading 147  
exceeded the negative control by 0.150 OD according to manufacturer's 148  
instruction. The test was reported with 96 % sensitivity and 100% specificity 149  
according to the manufacturer's instructions. 150

## 2.5 DNA extraction 151

All ELISA positive *Giardia* samples were subjected to DNA extraction 152  
using QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to 153  
manufacturer instructions with modification. Before extraction, samples were 154  
subjected to 5 cycles of freezing under liquid nitrogen and thawing at 95°C to 155  
break down the cyst walls. Extracted DNA was stored at -20°C for further PCR 156  
analysis [22]. 157  
158

## 2.6 PCR amplification and RFLP analysis 159

DNA molecular analysis was performed using nested PCR for the 160  
amplification of targeted region within the  $\beta$ -giardin gene. In the first PCR 161  
reaction primer pair; forward G7 (5'- 162  
AAGCCCGACGACCTCACCCGCAGTGC-3') and the reverse primer G759 (5'- 163  
GAGGCCGCCCTGGATCTTCGAGACGAC-3') were used to amplify a 753bp 164  
fragment [23]. The second PCR reaction amplifying 511bp fragment was 165  
performed using internal primers pair; forward G5 (5'- 166  
GAACGAACGAGATCGAGGTCCG-3') and the reverse G5 (5'- 167  
CTCGACGAGCTT CGTGTT-3') [24]. PCR cocktail for both reactions 168  
169

consisted of 10 $\mu$ M of each primer, 12.5 $\mu$ l of MasterMix (Promega, Germany), 170  
2 $\mu$ g BSA and 4 $\mu$ l of DNA templet in a total volume of 25 $\mu$ l. PCR was carried out 171  
in the thermocycler (Gene Pro Thermal Cycler, model TC-E-96G, China) under 172  
the following conditions: initial denaturation for 5 min at 96 $^{\circ}$ C, 35 cycles (20 sec 173  
at 95 $^{\circ}$ C, 30 sec at 53 $^{\circ}$ C and 45 sec at 72 $^{\circ}$ C) followed by a final extension of 7 174  
min at 72 $^{\circ}$ C. The second PCR cycling condition was like the first PCR run 175  
except the extension time was for 50 sec and final extension time was for 10 min. 176

The amplified products were digested using 10 U/ $\mu$ l of HaeIII (New 177  
England BioLabs, R0108L, UK) in a final volume of 20 $\mu$ l for 3 h at 37 $^{\circ}$ C. 178  
Digested pattern visualized on 3% agarose gel with ethidium bromide stain used 179  
for assemblage analysis, according to previous reports [24]. Appropriate 180  
negative and positive controls for each assemblage were employed. The test has 181  
a high sensitivity and specificity comparable to *ssrRNA giardia* gene [23]. To 182  
confirm the RFLP findings another PCR assay using assemblage A and B 183  
specific primers targeting the triose phosphate isomerase (*tpi*) gene was 184  
performed as described by Bertrand et al. [22]. 185

## 2.7 Statistical analysis 186

Data were stored in a database and analyzed using Epi-Info 7 (CDC, 187  
Atlanta, Georgia) and SPSS version16.0 [25]. The true prevalence was calculated 188  
using apparent prevalence, test sensitivity and specificity according to Rogan and 189  
Gladen [26] . The 95 % confidence interval was calculated for the prevalence. 190  
Chi-square analysis was used to test the association among the proportions, and 191  
the odds ratios were calculated. The dependent variables were: *Giardia* infection 192  
regardless of the assemblage type, *Giardia* assemblage A or B or E infections 193  
194

status, coded as 0 (negative) or 1 (positive) for each. A total of five variables 195  
(categories) were tested: species (donkey and horse), gender (male and female), 196  
age groups (less than and older than 5 years), climate (cool temperate rainy, cool 197  
desert, warm steppe, and warm temperate rainy), and season (spring, summer, 198  
autumn, and winter). The screening for the significant variables to be used in the 199  
final logistic regression was conducted using Chi-squared test. The variables that 200  
were statistically significant  $p < 0.2$  (two sided) were included in the 201  
multivariable model forward selection.  $P$  value of  $< 0.05$  was considered 202  
significant. 203

### 3. Results 205

Table 1 summarizes the true prevalence results. Of the 400 samples 206  
screened by ELISA test, 34 were positive for *Giardia* infection giving an 207  
apparent prevalence of 8.5% (95% CI; 5.8-11.2). The PCR-RFLP test confirmed 208  
*Giardia* assemblages in 30 of the 34 ELISA positive samples giving a true 209  
prevalence of 7.7% (95% CI; 4.8-10.1). Of the 30 positive animals, 18 had 210  
assemblage A (63% of infected equines and 68.4% of infected horses), 4 had 211  
assemblage B (13.3% of infected equines and 10.5% of infected horses) and 8 212  
had assemblage E (23.3% of infected equines and 21.1% of infected horses). 213  
PCR targeting *tpi* gene for assemblage A or B confirmed the results for RFLP. 214

Assemblage A was significantly more prevalent in equines compared to 215  
assemblages B and E ( $X^2 = 10.7$ , 2 d.f.,  $P = 0.005$ ). There was no significant 216  
difference between the prevalence of assemblages B and E ( $X^2 = 1.35$ , 1 d.f.,  $P =$  217  
0.24). There was no mixed infection with *Giardia* assemblages. The infection 218  
rates of *Giardia* regardless of assemblage type, assemblage B and E were 219

significantly ( $p < 0.05$ , Chi-square) higher in donkeys 14.8%, 2.7% and 5.5% 220  
compared to horses 5.8%, 0.6% and 1.2%, respectively. 221

The Chi-square results for the association of *Giardia* assemblages and 222  
analysis of evaluated risk factors are summarized in Table 2a, b and c. Only 223  
species and season were associated ( $p < 0.2$ ) with *Giardia* infection regardless of 224  
the assemblage type in equines (Table 2a). After forward selection, only the 225  
season autumn was significantly associated with *Giardia* infection (Table 3). 226  
Similarly, species and season were associated ( $p < 0.2$ ) with *Giardia* assemblage 227  
E infection (Table 2c) and after forward selection the model revealed that only 228  
spring season was associated (Table 3). Of the five variables/categories, age 229  
group and season were associated ( $p < 0.2$ ) with *Giardia* infection assemblage A 230  
in equines (Table 2b) and after forward selection the model had only winter 231  
season (Table 3). With regard to *Giardia* assemblage B infection, species was 232  
the only variable associated ( $p < 0.1$ ) with this assemblage in the univariable 233  
analyses only. 234

#### 4. Discussion 235

To the authors' knowledge, this is the first report evaluating *Giardia* 237  
prevalence and risk factors among horses and donkeys in different geographical 238  
and climate zones in Jordan. The results showed *Giardia* true prevalence rate 239  
among equines in Jordan to be 7.7% using ELISA and PCR-RFLP tests. Horses 240  
and donkeys living in the included locations were found to be infected with 241  
*Giardia* assemblages A, B and E. Also, the findings of the research reported here 242  
demonstrated significantly higher true prevalence of *Giardia* assemblages A 243  
(4.6%) when compared to assemblages B and E. Donkeys had higher infection 244

rate than horses, 14.8% and 5.8% respectively. This is considered the first report 245  
of the infection in donkeys with *Giardia* assemblages A and E worldwide, while 246  
the only publication on donkeys and *Giardia* [13] they do found only assemblage 247  
B. Seasons were associated with the infection with high odds during winter and 248  
spring and low odds during autumn depending on the type of *Giardia* 249  
assemblage. 250

Table 4a summarizes the previous reports on *Giardia* infection, 251  
regardless of assemblages and occurrence among equines worldwide. Previous 252  
reports on equines were all in horses except one [13] which included countries of 253  
the Middle East, Europe, and North and South America. Infection rates varied 254  
between 0 % in California and Nevada in the United States to 19.6% in Iraq 255  
employing different diagnostic tests. Most studies used the microscopy test (n = 256  
8) or direct immune fluorescent antibody test (3). The current work showed a 257  
lower true prevalence among horses (6.0%) in Jordan using ELISA and PCR 258  
tests than most reported rates. The tests used had a high sensitivity and 259  
specificity using one sample [23, 27]. In addition, the nature of horse husbandry 260  
with restricted movement, limited grazing and low density, might have played a 261  
role in lowering the infection rate compared to other parts of the world. 262

In Jordan, donkeys are exposed to humans, dogs, cats and wild life either 263  
directly or indirectly. During the grazing season, donkeys are an integral 264  
component of small ruminant (sheep and goats) flocks providing means of 265  
transportation for the shepherd and carrying their tools. Also, in some flocks, 266  
donkeys are equipped with a bell around its neck and used as a small ruminant 267  
flock leader followed by the whole flock in travelling, replacing the head ram. 268  
Thus donkeys, especially during the grazing season (spring and summer) are 269

relatively more exposed to environmental contamination with *Giardia* from 270  
infected sheep, goats, dogs and cats. On the other hand, movement of horses is 271  
commonly more restricted as they are kept in stables where they are exposed to 272  
dogs, cats and wild animals in addition to humans but less so with sheep and 273  
goats in contrast with donkeys. 274

Reports evaluating *Giardia* assemblages in equines are available from six 275  
countries in Europe, one country in South America and China (Table 4b). Four 276  
studies reported the occurrence of assemblage A in six European countries, 277  
Colombia and China. The contribution of both Assemblages A and B to infection 278  
ranged from 0 to 50 % while that of assemblage E ranged from 0 to 100%. The 279  
current results demonstrated higher proportions of assemblage A in infected 280  
horses (68.4%) and donkeys (46%). The prevalence rate in the paper presented 281  
here is considered high when compared with the prevalence rate of *Giardia* in 282  
horses worldwide (Table 4b). 283

Other studies reported that B as the dominant assemblage in horses [7, 12, 284  
28]. The variability in the dominant assemblage infecting equines in different 285  
countries/areas, especially the presumed zoonotic variants (A and B), might be 286  
affected by environmental factors, season and management factors including the 287  
contamination of the pasture and/or water resources with human and animal 288  
waste. Zhang et al. [13] study on donkeys in China identified 4, 1 and 3 subtypes 289  
of *Giardia* assemblage B only. 290

Most studies reported the occurrence of assemblage E in horses (Table 291  
4b). The percent of assemblage E among evaluated samples ranged from 0% to 292  
100%. In Jordan, among infected samples, 21% of the horses and 36% of the 293

donkeys had assemblage E with percentages ranked second after assemblage A 294  
and ahead of assemblage B and similarly the prevalence (Table 1). Assemblage E 295  
has been reported as the dominant assemblage in cattle and sheep [29-31]. Thus, 296  
in Jordan, assemblage E is expected to occur more in equines especially donkeys 297  
where most (98%) of domestic animal populations are sheep and goats which 298  
have a close association with donkeys. 299

Although this study had the limitation of being conducted during one year 300  
period, seasonal influence on the *Giardia* infection rate, regardless of 301  
assemblages, was clear in this paper. The prevalence was highest in the winter 302  
(13%) and spring (10%) compared to the summer (6%) and autumn (1%). In 303  
Jordan, spring and summer are the main grazing seasons. The increase in the 304  
odds of infection in winter can be explained by the contamination of the barns, 305  
water and the pasture with infective *Giardia* cysts from infected animals. This is 306  
exacerbated by overcrowded conditions and the rain water flushing the cysts into 307  
the environment, spreading them over a wider area exposing the infection to 308  
other animals. 309

In the United Kingdom, it is believed that transmission of *Giardia* is 310  
increased by heavy rain [32]. In Jordan, the winter, autumn and spring seasons 311  
are wet while the summer is dry with reported shortages of water. Although the 312  
effect of rain falls in autumn on cyst dispersal is minimal compared to winter and 313  
spring. This may be explained by soil aridity caused by the long hot dry summers 314  
which kill the infective cysts and dries out the soil to a significant depth 315  
facilitating the absorption of the precipitation occurring during the autumn 316  
season washing down the cysts from the topsoil without significant runoff. Thus 317  
*Giardia* contamination in the environment is reduced. 318

The prevalence of assemblage A is significantly higher in winter (6.8%) 319  
while assemblage E was highest in the spring, this can be explained by the source 320  
of the infection. As assemblage A presumed to be zoonotic with a winter peak 321  
concomitant with the rainy season and the highest rainfall in Jordan. This may be 322  
due to the contamination of water resources with the human and animal waste. 323  
Conversely, assemblage E which is not considered as zoonotic is more common 324  
in hoofed farm animals [29-31]. The peak prevalence of this assemblage is in the 325  
grazing season (spring) and may indicate the role of grazing animals in 326  
contaminating the environment. Also, giardiasis is more common in young 327  
animals as age resistance usually occurs over time. Spring is the lambing and 328  
kidding season with the largest population of susceptible young lambs and kids 329  
which would potentially shed large numbers of *Giardia* cysts leading to the 330  
consequent high environmental contamination in the spring. 331

Our study was conducted on apparently healthy adult animals which 332  
resulted in the limited effect of age on the prevalence. On the other hand, it is 333  
important to study these subclinical animals which serve as reservoir for *Giardia* 334  
that infect humans and other animals. In addition, although samples were 335  
collected from 4 different areas representing variable climate zones, there was 336  
only a limited effect on *Giardia* prevalence. The relative proximity between 337  
these areas (with 70 km radius), the free movement of animals and trading, and 338  
transhumance type of grazing adopted in the Middle East could all explain the 339  
minimal area effect on the prevalence. 340

Although using  $\beta$ -giardin PCR-RFLP is a sensitive technique in detecting 341  
*Giardia* assemblages, more work is needed to detect sub-assemblages to confirm 342  
the zoonotic nature of the assemblages found compared to the sub-assemblages 343



found infecting humans in Jordan. In addition, the absence of mixed assemblages 344  
infection in our study might need confirmation using other gene target. Finally, 345  
wider studies including other animal species and drinking water quality are 346  
needed to document the transmission dynamic of *Giardia* in Jordan. 347

## 5. Conclusions 349

The current study documented for the first time the prevalence of *Giardia* 350  
including assemblages A, B and E in equine in Jordan and assemblages A and E 351  
in donkeys worldwide. The dominance of the presumed zoonotic assemblage A 352  
in this study may indicate the role of equines as a source of environmental 353  
contamination with *Giardia* with all impact on human and animal health in 354  
Jordan. 355

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Table 1	479
True prevalence of <i>Giardia</i> assemblages detected in equines (n=400) using the	480
ELISA test and confirmed by PCR-RFLP analysis targeting Beta giardin loci and	481
digested with HaeIII enzyme during September 2014 to August 2015.	482

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.,  $P = 0.005$

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Table 2a 486  
 Univariate association between potential risk factors and *Giardia* infection 487  
 positivity regardless of the assemblage type, by ELISA and PCR-RFLP tests 488  
 among 400 equines during the period September 2014 to August 2015 in Jordan 489

Variable	Category	Coding	Total No.	+ve % (n = 30)	<i>P</i>
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)	

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Table 2b 491  
 Univariate association between potential risk factors and *Giardia* Assemblage A 492  
 infection positivity by ELISA and PCR-RFLP tests among 400 equines during 493  
 the period September 2014 to August 2015 in Jordan 494

Variable	Category	Coding	Total No.	+ve % (n = 18)	<i>P</i>
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)	

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Table 2c 497  
 Univariate association between potential risk factors and *Giardia* Assemblage E 498  
 infection positivity by ELISA and PCR-RFLP tests among 400 equines during 499  
 the period September 2014 to August 2015 in Jordan 500

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)	

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Table 3	502
Multivariable logistic regression models of factors associated with <i>Giardia</i>	503
Assemblages A, B and E infection detected by ELISA and PCR-RFLP tests	504
among 400 equines (horses and donkeys) during the period September 2014 to	505
August 2015 in Jordan	506

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

<sup>a</sup>Likelihood ratio of chi-squared (LRX<sup>2</sup>): 106.6 on one degree of freedom (d.f.), 507

<sup>b</sup>LRX<sup>2</sup>: 131.6 on one df, <sup>c</sup>LRX<sup>2</sup>: 69.9 on one d.f. 508

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Table 4a	510
Previous work on <i>Giardia</i> infection regardless of assemblages' type among horses worldwide and donkeys from Jordan only	511
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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]

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Table 4b	515
Previous work on <i>Giardia</i> assemblages' infections among horses worldwide and	516
donkeys (from Jordan only)	517

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

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## 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