

Prevalence of *Giardia spp.* and *Cryptosporidium spp.* in horses and animal handlers in some areas in Baghdad city

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Abstract

This study was conducted to detect the zoonotic intestinal protozoa *Giardia spp.*, *Cryptosporidium spp.* infection in horses and animal handlers at Baghdad city (Al-Zawraa Zoo, Iraqi Equestrian Federation (IFE), Equestrian and Al-Dowanem), during the period 1/November/ 2014 to 31/April /2015, from both sexes one hundred eighty stool samples (92 horses, and 88 humans) were examined by direct wet smear, Lugol's iodine smear and modified Ziehl-Neelsen stain. In horse the results showed that the infection rate of *Giardia spp.* was 4.45% by direct wet and Lugol's iodine smears. The higher infection rate 12.5% was recorded in the age group < 1-3 years and the females showed a higher infection rate 6%. In Equestrian were recorded the high infection rates 8% without significant $P>0.05$. While in animal handlers was showed a total infection rate 35.22%, the higher infection rate 45% was recorded in the age group <20 years and the high infection rates were recorded 36.36% in males. while the higher infection rate 50% was found in Iraqi Equestrian Federation and all group show no significant $P>0.05$. The results were showed that the total *Cryptosporidium spp.* infection rate was 64.13% by MZNS in horse. The higher infection rate recorded 70% in age group 12-15<years, while higher infection rate were recorded in males 71.43% with no significant between the age and sex $P>0.05$. Al-Zawraa Zoo was recorded the highest infection rate 85.18% with different significant $P<0.01$. *Cryptosporidium spp.* in animal handlers showed total infection rate 47.72%, the higher infection rate 77.77% was recorded in the age group 40-50< years and the group show significant $P<0.01$, with high infection rates 50% recorded in males. The higher infection rate 59.9% was recorded in Al-Dowanem areas. In ELISA for detection of *Cryptosporidium spp.* and *Giardia spp.* parasites of animal handlers, the result showed that the total infection rate was 60.23%, the higher infection rate 72.22% was found in the age group 40-50< years with different significant $P<0.01$. A high infection rate 63.64% was found in females and the Equestrian area was showed the higher infection rate 76.47% without significant $P>0.05$.

Key word :horse, animal handlers, *Giardia*, *Cryptosporidium*, Equestrian

انتشار طفيليات الجيارديا والأبواغ الخبيثة في الخيول ومربيها في بعض مناطق مدينة بغداد

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الخلاصة

هدفت هذه الدراسة التحري عن الإصابة بالالوي المعوية الطفيلية المشتركة (الأبواغ الخبيثة والجيارديا) في الخيول ومربيها في مدينة بغداد (حديقة حيوان الزوراء، الاتحاد العراقي للفروسية، إسطبلات نادي الفروسية، ومنطقة الدوانم) للمدة من 1/ تشرين الثاني/ 2014 ولغاية 31/ نيسان 2015 من خلال جمع 180 عينة براز (92 خيول و88 مربي الخيول) فحصت بالمسحة الرطبة المباشرة وصبغة الايودين، صبغة زيل نلسن المحورة. وأظهرت نتائج الكشف ان نسبة الإصابة الكلية بطفلي الجيارديا في الخيول 4.45% باستعمال المسحة الرطبة المباشرة وصبغة

الايودين، وسجلت الفئة العمرية >1-3 سنة اعلى نسبة إصابة 12.5%، وكانت نسبة الإصابة في الإناث 6% هي الأعلى، وسجلت نسب إصابة 8% في إسطبلات نادي الفروسية، وسجلت نسبة إصابة كلية للطفلي بلغت 35.22% في مربي الخيول وكانت اعلى نسبة 45% في الفئة العمرية <20 سنة، وقد بلغت نسبة الإصابة 36.36% الأعلى في الذكور، وكانت اعلى نسبة إصابة 50% في الاتحاد العراقي للفروسية. وأظهرت النتائج ان النسبة الكلية للإصابة بطفلي الأبواغ الخبيثة 64.13% في الخيول باستعمال صبغة زيل نلسن المحورة، إذ سجلت اعلى نسب إصابة 70% في الفئة العمرية 12-15 > سنة، وسجلت نسبة الإصابة الأعلى في الذكور 71.43%، وسجلت حديقة حيوان الزوراء اعلى نسبة 85.18% مقارنة ببقية مناطق الدراسة، وفي حين بلغت نسبة الإصابة الكلية 47.72% للمربين وكانت النسبة الأعلى للإصابة 77.77% في الفئة العمرية 40-50 > سنة، وبلغت اعلى نسبة للإصابة في الذكور 50%، وسجلت منطقة الدوانم اعلى نسبة 59.9%. أما باستخدام مقاييس الممتز المناعي المرتبط بالإنزيم لطفلي الأبواغ الخبيثة والجيارديا في مربي الخيول، فقد بلغت نسبة الإصابة الكلية 60.23%، وسجلت اعلى نسبة إصابة 72.22% في الفئة العمرية 40-50 > سنة وسجلت الإناث اعلى نسبة إصابة 63.64% من الذكور وكانت اعلى نسبة إصابة 76.47% في إسطبلات نادي الفروسية.

الكلمات المفتاحية: الخيول، المربين، الجيارديا، الأبواغ الخبيثة، الفروسية.

Introduction

Giardia and *Cryptosporidium* are a common zoonotic protozoan parasite that causes disease in humans, and animals (1, 2). With a worldwide distribution and it can cause severe and life-threatening diarrhea in an immunocompromised host (3). *Giardia* has been found in more than 40 animal species (4), The zoonotic potential of *Giardia* in horses remains largely an unexplored issue. If zoonotic transmission is possible, farm, recreational as well as wild horses may constitute a source for human infection either directly or via watersheds. Although (5) has been reported the presence of *Giardia* in horses with diarrhea. also horses infected with *Giardia* and *Cryptosporidium* show clinical signs of diarrhea, colic, lethargy and anorexia but rarely. Other subclinical symptoms such as a reduction in growth failure to thrive, as shown with livestock (6), it is a common cause of diarrhea in humans and animals worldwide. It can be acquired by ingestion of oocysts that were excreted in the feces of infected individuals. So that the major clinical sign of Cryptosporidiosis in foals is diarrhea, but most *Cryptosporidium* infections in adult horses are asymptomatic (7). Cryptosporidiosis in horses was initially described in 5 immuno-deficient Arabian foals. Subsequently, however, Cryptosporidiosis has been reported in immuno-competent horses worldwide (7) Cryptosporidiosis is reported to infect persons in 106 countries (8) while (9) mention the infection with parasite 75%. in Santa Maria-RS, Brazil. other studies have revealed the prevalence of *Giardia* in horses to range from <3.2% in horses used for backcountry recreation in California (10) to 4.6% in horses and mules used as pack stock in the Sierra Nevada Range (11), the human giardiasis has been reported to range from 2% to 7% in developed countries and 20%-30% in the developing countries, with more than 200 million people in Asia, Africa and Latin America have symptomatic infections with about 50,000 cases reported to being affected every year (12, 13). Transmission of *Giardia* and *Cryptosporidium* is via the fecal-oral route, either indirectly through contaminated water or food, or directly from person-to-person or animal-to-person contact. Also transmission can occur from person-to-person, from animal-to-person, animal-to-animal, (14). Also (15) refer to possible air-borne infection has been established by Many accidental infections can occur among researchers or laboratory workers during handling of infected samples or laboratory animals.

Material and Methods

- **Samples collection:** Single fecal samples were collected and processed for the detection of trophozoite or cyst stages of intestinal parasites. In total Ninety two fecal samples (92 horses and 88 animal handlers) both sexes clinically healthy were collected by using clean screw cup and transport to the zoonotic unite of Veterinary Medicine College/ Baghdad University. The horse samples were tested by the traditional methods (Direct wet smear, Lugol's iodine and Modified Ziehle Nelseen stain), while the human samples were divided into two parts the first one for the traditional tests (Direct wet smear, Lugol's iodine and Modified Ziehle Nelseen stain), and the second part was kept in deep freezer at -20°C for detected of *Cryptosporidium* spp. and *Giardia* spp. parasites by ELISA. Direct wet smear, Lugol's iodine using for detected *Giardia* spp. for the identification of cyst in human and horse. While for detected *Cryptosporidium* spp. Modified Ziehle Nelseen stain for the identification of red-pink oocysts of acid-fast parasites in human and horse (16). *Giardia* Ag Combo/ Crypto ELISA (Fecal) U.S.A. Diagnostic Automation, INC. Cat#8310-3. using for detection the two parasite in human.
- **Statistical analysis:** The data analyzed by used SPSS for different groups by using Chi square at levels 0.05 and 0.01 (17).

Results

- Detection of *Giardia* spp. and *Cryptosporidium* spp. in animal handlers by using direct wet smear, Lugol's iodine and Modified Ziehle Nelseen Stain.
- Infection rate of *Giardia* spp. and *Cryptosporidium* spp. according to the age: The higher infection rate 9(45%) of *Giardia* spp. in animal handlers was founded in age group under 20 years, without significant difference ($P \geq 0.05$). while higher infection rate 14 (77.77%) of *Cryptosporidium* spp. in animal handlers was recorded in age group 40-50< years with a significant difference ($P < 0.01$). (Table1).

Table (1) The infection rates of *Giardia* spp and *Cryptosporidium* spp. according to the ages of animal handlers

Age (years)	No. of samples examined	<i>Giardia</i> spp.	Percentage (%)	<i>Cryptosporidium</i> spp.	Percentage (%)
		No. of positive		No. of positive	
<20	20	*9	45	**12	60
20->30	26	7	26.92	10	38.46
30->40	24	9	37.5	6	25
40-50<	18	6	33.33	14	77.77
Total	88	31	35.227	42	47.72
Chi sq			1.70		12.24
p			0.63		0.006

*($P \geq 0.05$) **($P < 0.01$)

- Infection rate of *Giardia* spp. and *Cryptosporidium* spp. according to the sex in animal handlers: Table (2) showed a no significant difference ($P \geq 0.05$) between both sexes with high infection rate of *Giardia* spp. 36.36% in females. While the infection rate of *Cryptosporidium* sp. in males with 50% rates of infection and no significant difference ($P \geq 0.05$) was found.

Table (2) The infection rates of *Giardia* spp. and *Cryptosporidium* spp. according to the sex in animal handlers

Sex	No. of samples examined	<i>Giardia</i> spp.	Percentage (%)	<i>Cryptosporidium</i> spp.	Percentage (%)
		No. of positive		No. of positive	
Males	66	23	34.84	33	50
Females	22	8	36.36	9	40.90
Total	88	31	35.227	42	47.72
Chi sq			0.019		0.54
p			0.89		0.45

*($P \geq 0.05$) **($P \geq 0.05$)

- Infection rate of *Giardia spp.* and *Cryptosporidium spp.* in animal handlers according to the areas: A different infection rates of *Giardia spp.* were recorded in different areas of the present study without significant difference ($P \geq 0.05$). A high infection rate was 50% found in Iraqi Equestrian Federation. Also a different infection rates of *Cryptosporidium spp.* in the different areas which were high rates was recorded in Al-Dowanem 59.09% without significant difference ($P \geq 0.05$) as show in (Table 3).

Table (3) The infection rates of *Giardia spp.* and *Cryptosporidium spp.* in animal handlers according to the areas of the study

Areas	No. of samples examined	<i>Giardia spp.</i>	Percentage (%)	<i>Cryptosporidium spp.</i>	Percentage (%)
		No. of positive		No. of positive	
Al-Zawraa Zoo	22	8	36.36	6	27.27
Iraqi Equestrian Federation	14	7	50	6	42.57
Equestrian	30	6	20	17	56.66
AL-Dowanem	22	10	45.45	13	59.09
Total	88	31	35.227	42	47.72
Chi sq			5.40		5.92
p			0.14		0.11

*($P \geq 0.05$) **($P \geq 0.05$)

- Detection of the infection of *Cryptosporidium spp.* and *Giardia spp.* in animal handlers by using ELISA.
- Infection rate according to the age of animal handlers: There was no significant difference ($P \geq 0.05$) in the infection rate of *Cryptosporidium spp.* and *Giardia spp.* recorded in the different age groups of animal handlers by ELISA, which the higher rate was 13 (72.22%) in the age group 40-50< years. (Table4).

Table (4) Total infection rates of *Giardia spp.* and *Cryptosporidium spp.* according to the age in animal handlers by ELISA

Age (years)	No. of samples examined	No. of positive	Percentage (%)
<20	20	12	60
20->30	26	15	67.69
30->40	24	13	54.17
40-50<	18	13	72.22
Total	88	53	60.23
Chi sq			1.51
p			0.67

*($P \geq 0.05$)

- Infection rate according to the sex in animal handlers by ELISA: According to the sex, the females was showed a higher infection rate of *Cryptosporidium spp.* and *Giardia spp.* by ELISA 14 (63.64%) with no significant difference ($P \geq 0.05$). (Table5).

Table (5) Total infection rates of *Giardia spp.* and *Cryptosporidium spp.* according to the sex by ELISA in animal handlers

Sex	No. of samples examined	No. of positive	Percentage (%)
Males	66	39	59.09
Females	22	14	63.64
Total	88	53	60.23
Chi sq			0.14
p			0.70

*($P \geq 0.05$)

- Infection rate according to the areas in animal handlers by ELISA: Table (6) was showed the infection rate of *Cryptosporidium spp.* and *Giardia spp.* by ELISA was higher 17 (76.47%) in Equestrian among other areas, without significant difference ($P \geq 0.05$).

Table (6) Total infection rates of *Giardia spp.* and *Cryptosporidium spp.* according to the area by ELISA in animal handlers

Area	No. of samples examined	No. of positive	Percentage (%)
Al-Zawraa Zoo	22	13	59.09
Iraqi Equestrian Federation	14	9	64.29
Equestrian	30	17	76.47
AL-Dowanem	22	14	63.64
Total	88	53	60.23
Chi sq			0.37
p			0.94

*($P \geq 0.05$)

- Detection of *Giardia spp.* and *Cryptosporidium spp.* in horses: By using direct wet smear, Lugol's iodine and Modified Ziehl Neelsen Stain.
- The infection rate according to the age of the horse: Table (7) was showed the high infection rate 2 (12.5%) of *Giardia spp.* in age groups <1->3 years, without significant difference ($P > 0.05$). while A higher infection rate 14 (70%) of *Cryptosporidium spp.* in 12-15<years, also without a significant difference ($P > 0.05$).

Table (7) The infection rates of *Giardia spp.* and *Cryptosporidium spp.* in horses according to the age

Age (years)	No. of samples examined	<i>Giardia spp.</i>	Percentage (%)	<i>Cryptosporidium spp.</i>	Percentage (%)
		No. of positive		No. of positive	
<1->3	16	2	12.5	10	62.5
3->6	24	0	0	16	66.66
6->9	20	0	0	11	55
9->12	12	1	8.33	8	66.66
12-15<	20	1	5	14	70
Total	92	4	4.35	59	64.13
Chi sq			5.03		1.14
p			0.28		0.88

*($P \geq 0.05$)

**($P \geq 0.05$)

- The infection rate according to the sex of the horses: In table (8) the higher infection rate 3(6%) of *Giardia spp.* was recorded in the females, without significant differences ($P > 0.05$). While the *Cryptosporidium spp.* infection rate showed a higher infection rate 30 (71.43%) was recorded in males, with significant differences ($P < 0.01$).

Table (8) The infection rates of *Giardia spp.* and *Cryptosporidium spp.* in horses according to the sex

Sex	No. of samples examined	<i>Giardia spp.</i>	Percentage (%)	<i>Cryptosporidium spp.</i>	Percentage (%)
		No. of positive		No. of positive	
Males	42	*1	2.38	** 30	71.43
Females	50	3	6	29	58
Total	92	4	4.35	59	64.13
Chi sq			0.71		1.78
p			0.39		0.18

*($P > 0.05$) **($P < 0.01$)

- The infection rate according to the areas of the study: Areas of the study were showed a different infection rates of *Giardia spp.* The higher infection rate 8% was recorded in Equestrian area with no significant difference ($P>0.05$). while a high infection rates 85.18% of *Cryptosporidium spp.* were recorded in Al-Zawraa Zoo with significant difference ($P<0.01$). (Table 9).

Table (9) The infection rate of *Giardia spp.* and *Cryptosporidium spp.* in horses according to the areas of the study

Areas	No. of samples examined	<i>Giardia spp.</i>	Percentage (%)	<i>Cryptosporidium spp.</i>	Percentage (%)
		No. of positive		No. of positive	
Al-Zawraa Zoo	27	2	7.40	23	**85.185
Iraqi Equestrian Federation	25	0	0	16	64
Equestrian	25	2	8	10	40
Al-Dowanem	15	0	0	10	66.66
Total	92	4	4.35	59	64.13
Chi sq			3.22		11.57
p			0.35		0.009

*($P>0.05$) **($P<0.01$)

Discussion

Our study result in horsemen is nearly similar that was recorded by [18,19] had shown the prevalence between 1-30%. And the reason of this high prevalence may be attributed to the low immunity against various pathogens as these age groups are comparatively less resistant to diseases as described in a previous study (20). By the other hand the result in *Giardia* is agreement with (21) that recorded 24.65% and disagreement with 11.7% in Morocco (22). while in *Cryptosporidium* our result agree with (23) reported 43.7% and disagree with (24, 25) were mention 14.3% and (20.52%) respectively. According to the sex the results of the present study of *Giardia* were disagree with (26) who reported that males had a higher *Giardia* infection rate than females, and (27) found males had lower infection rates 1.51% in males than in females 2.18% and agree with (28) were found 65% (81/124) males and 35% (43/124) females. Also (29) seated there were 254 (41.9%) males and 352 (58.1%) females while the infection rates of *Cryptosporidium spp.* disagree with (30) that recorded 5.26% and 1.71% in males and females respectively. The rate of infection in males was higher than in females in *Cryptosporidium spp.* While the distribution of the areas showed agreement with (31). That recorded the infection rate with giardiasis in developing countries range from 5-43% also The rate of *Giardia* was nearly similar those 44.59%, 35.89%, and 62.2% recorded in Kirkuk, Erbil in Iraq and in Egypt respectively by (32, 33, 34) it was not agree with those of 11.4% and 17.1% recorded in Libya and Brazil respectively by (35, 36). While *Cryptosporidium* rate 47.72% was disagree with 7.60% in Kirkuk and 10% recorded in Kuwait (21, 37). And the other reason could be related to a number of factors such as poor health hygiene and toilet training, overcrowding, low socioeconomic status and climatic conditions (38) also might be due to that males are mostly outside their houses and are mostly exposed to fecal transmitted parasites. As well as the age, nutritional status, predisposing illness, and previous exposure, safety of water consumption from water supplies. In addition to type of diagnostic techniques, size of samples (21) By ELISA our study agree with (26) found that in comparison study between microscopic stool examination (gold standard) and ELISA test for diagnosis of *Giardia*, The prevalence of infection was 50.0% (42/84) by direct examination and 65.5% by ELISA (55/84) Of 84 stool samples, 42 were positive and 42 were negative for *Giardia spp.* stages by using microscopic stool examination. All the

samples that were positive by microscopy were positive by ELISA test and 13 samples that were negative by microscopy were positive by ELISA test. These a good dissuasion for our result also, and the sensitivity of the ELISA test was different from kit to other as well as the technique. Therefore other studies found a sensitivity and specificity of 96.4%, 80.8% and 98%, 92% respectively of *Giardia* antigen in stool. (39) While our result was not agree with (40) reported (12.4%, 6% and 14.78% respectively). and (21) with 13.13% while agree with the sensitivity of ELISA test 53.27% in Kirkuk city (21) and 76.4% that recorded in Duhok province by (41). Equine *Giardia spp.* and *Cryptosporidium spp.* was previously reported in other parts of the world and it is believed that is neither associated with the age of the horses nor with mode of life (10) and our study agreement with (9) in Brazil that referred that the infection with *Cryptosporidium* in foals below 2 years of age was 83.3% (15/18); 71% (22/31) in young foals aged between 3-5 years of age, and 80% (12/15) in adult horses. Studies have revealed the prevalence of *Giardia* in horses range from <3.2% in horses used for backcountry recreation in California (10) to 4.6% in horses used as pack stock in the Sierra Nevada Range (11) and the study disagreement to 20% in Canadian farms (42) and (43) found *Cryptosporidium spp.* infection 9.4%, and with (21) which recorded 0% infection with *Giardia spp.* in Jockey Club of Santa Maria. according to the sex the infection of *Cryptosporidium spp.* our result disagreement with reported from Germany, Poland and Texas and Colorado, USA, which the infection rates ranged from 0.33-9.4% (44). While agreement with reported by researchers from Canada and other localities of Louisiana, Colorado and Texas, USA which infection rates ranged from 17–100% (42, 45, 46, 47). While in *Giardia spp.* disagreement with (42) relatively high rates of giardiasis among foals 17-35% and agreement with (48) recorded 0.66% as well as with (47) were referred that the infection rate in lactating mares 1.9-27.8%. The prevalence according to areas of *Giardia* in horses has been shown to vary considerably 0-25% between geographical locations (11, 42), so our study agreement with (11) that recorded the infection of *Giardia* 4.6%. While the infection rate of *Cryptosporidium spp.* in our study agreement with 75% (21) and disagree with 26.66% that recorded by (49) in mashhad/Iran. These results show that *Cryptosporidium spp.* is widely disseminated in this population, and it can represent an important source of infection for the population in the region. age and physiological status of the animal(47). Also the prevalence of *Giardia* in horses in other parts of the world. Wild horses, or those horses kept on farmed or back country properties for recreational and pack stock purposes may be acting as sources of contamination of *Giardia spp.* for the environment, especially around watershed areas. Direct contact with working and recreational horses may also constitute a source of infection for humans (50).

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