

Detection of *Cryptosporidium* and *Giardia doudenalis* in equines in Nineveh, Iraq

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Abstract

A total of 107 fecal samples of equines from different regions in Nineveh, were collected from January 2007 till December 2007 and examined for *Cryptosporidium sp.*, and *Giardia doudenalis* by using different methods (wet mount, flotation, lugol's iodine, modified Ziehl Nelsen (hot) and Giemsa stain Just for *Giardia doudenalis*. The animal age examined ranged from 4 to 10 years. The total prevalence of *cryptosporidium sp.* was 27.10% (29 out of 107), while the prevalence of *Giardia doudenalis* was 19.63% (21 out of 107). This study represents the first trial to explore cryptosporidiosis, giardiasis in equines as in Nineveh there is no survey of these intestinal protozoa.

Keywords: *Cryptosporidium sp.*; *Giardia doudenalis*; Intestinal protozoa.

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كشف طفيلي البويغات الخبيثة والجيارديا في الخيول في نينوى، العراق

انتصار توما بطي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم جمع (107) عينة براز من الفصيلة الخيلية من مناطق مختلفة من محافظة نينوى للفترة من كانون الثاني 2007 الى كانون الأول 2007 وذلك للتحري عن وجود كلا من طفيلي الابواغ الخبيثة والجيارديا باستخدام طرق مختلفة: (المسحة الرطبة، طريقة التطويق، صبغة الايودين، والصبغة الصامدة للحمض المحورة (الحارة)، وصبغة كميزا لتوضيح طفيلي الجيارديا. تراوحت أعمار الحيوانات المفحوصة من 4 سنوات الى 10 سنوات. بلغت نسبة الإصابة الكلية لطفيلي الابواغ الخبيثة 27,10% (29 من 107) بينما بلغت نسبة الإصابة الكلية لطفيلي الجيارديا 19,63% (21 من 107). تناولت الدراسة أهمية الإصابة بكلا الطفيليين في الفصيلة الخيلية وتعتبر أول محاولة للتحري عن هذين الطفيليين المعويين في الخيول.

Introduction

Cryptosporidium parvum and *Giardia doudenalis* are recognized as primary enteric pathogens in animals and human (1). The severity of enteric disease by either of these parasites ranged from self-limiting to a fulminating diarrhoea. These parasites can be transmitted by ingestion of contaminated food or water (2).

Numerous studies have been carried out in our locality concerning *Cryptosporidium sp.*, in farm animals, Baker recorded the infection with cryptosporidiosis in calves (3),

Albakry (4) in goats *capra hircus* in Nineveh governorate in lambs (*ovis aries*) by khalil (5), while in horses *Equus caballus* there is no study that deals with this parasite, in comparison to data for bovine and human cryptosporidial infection.

There is a few information concerning equine cryptosporidiosis, although this disease has been reported in various regions of the world, which is connected with diarrhoea in foals (6,7).

Furthermore, there is an important role of equine *Cryptosporidium* infection as a source of zoonotic disease

(8). Some animals may carry *Giardia* organisms and animals appear healthy with no signs of disease, such hosts have a high potential rate for spreading the parasite. Infected animals can develop bloody diarrhoea or mucous stool often accompanied by gas production (9).

The first occurrence of *Giardia sp.*, was reported in horses in the Czech Republic by (10). Therefore these study deals with those intestinal protozoa (*Cryptosporidium sp.*, and *Giardia sp.*) in horses it is considered the first trial in our locality in equine family. The aim of this study was to investigate the prevalence of cryptosporidiosis and giardiasis in horses suffering from diarrhoea and those healthy, and also to determine the possible correlation between *cryptosporidium* and other parasite producing diarrhoea mainly *Giardia* in horses in Nineveh governorate.

Materials and methods

The study was carried out through a period of 12 months from January 2007 to December 2007. Atotal of 107 faecal samples of horse taken from both sex of equines family were collected from different regions in Nineveh governorate, samples were stored in clean plastic container covered with 2.5% potassium dichromate solution of preservation. Samples were tightly closed and stored at 4 °C till examination.

The following methods were used for detection of *Cryptosporidium* oocysts and *Giardia* cysts in faeces samples were stained with the following: wet mount (lugol's Iodine 1% and 5%) (11), and sheather's flotation technique were used for primary diagnosis of oocyst and cyst. Modified Ziehl- Nelsen stain (hot method) for further investigation for *cryptosporidium* oocysts (12). Giemsa stain was used for distinguishing *Giardia* cyst (13). The oocysts of *cryptosporidium* and cyst of *Giardia* were measured and identified as described by (5,14).

Chi-square X², Fisher test F were used to compare the frequencies of positive and negative specimens (15).

Results

Twenty nine positive samples out of 107 (27.10%) for *cryptosporidium* infection, and 19.63% for *Giardia* infection. The percentage of infection with *Cryptosporidium* 27.10%, and 19.63% with *Giardia* among the total examined specimens.

Although *Cryptosporidium sp.*, and *Giardia doudenalis* were detected through out the year, table (1) shows, the highest rate of *Cryptosporidium* infection was found in April (55.56%), followed by September (50.0%), then October (42.86%), March (36.36%), (25%) in June, July, May (23.08%), December (15.38%), August (20.0%), January (14.29%) (12.5%) in November and lowest rate of

infection of *Cryptosporidium* was (10.0%) in February. No significant difference in the rate of infection between the months of the year was found.

Also the highest rate *Giardia* infection was found in March (45.45%), and the lower rate in January and July (0.0%). There was no significant difference in the infection rate between the months of the year for *Cryptosporidium* and *Giardia*.

Table (2) shows the distribution regarding to age group, the highest rate of infection was found among 5-6 years (34.38%), followed by the group of 0 ≤ 4 years (31.58%) and (27.59%) in group 7-8 years old, and the lowest rate of infection among the group of 9-10 years old was (14.81%).

Table 1: Distribution of *Cryptosporidium sp.*, and *Giardia doudenalis* according to the months of the year.

months of the year	No. of samples	<i>Cryptosporidium</i>		<i>Giardia</i>	
		+ve samples	%	+ve samples	%
January	7	1	14.29	0	0.0
February	10	1	10	2	20.0
March	11	4	36.36	5	545.45
April	9	5	55.56	3	33.33
May	13	3	23.08	1	7.69
June	12	3	25.0	2	16.67
July	4	1	25.0	0	0.0
August	5	1	20.0	1	20
September	8	4	50.0	2	25.0
October	7	3	42.86	2	28.57
November	8	1	12.5	2	25.0
December	13	2	15.38	1	7.69
Total	107	29	27.10	21	19.63
P		No significant		No significant	

Table 2: Distribution of *Cryptosporidium sp.*, and *Giardia doudenalis* according to the age.

Age / year	No. of samples	<i>Cryptosporidium</i>		<i>Giardia</i>	
		+ve samples	%	+ve samples	%
0 ≤ 4	19	6	31.58	3	15.79
5-6	32	11	34.38	5	15.63
7-8	29	8	27.59	7	24.13
9-10	27	4	14.81	6	22.22
Total	107	29	27.10	21	19.63
P		No significant		No significant	

Statistical analysis shows no significant difference between age groups, for *Cryptosporidium*, while in *Giardia doudenalis* the rate of infection among age groups were high at 7-8 (24.13%) was followed by 9-10 years old group (22.22%) then the rate of infection among 0 ≤ 4 age group

was (15.79%) and the lowest rate of infection among 5-6 age group was (15.63%) no significant difference between age group for *Giardia*.

Ocular micrometer calibrated lens was used for measuring the oocyst of *Cryptosporidium sp.*, the results revealed that the average of measurement of oocyst was 4.9-6.2 μm which were diagnosed by sugar flotation method and modified Ziehl-Nelsen method the oocysts appears as bright rose - pink (Fig. 1). Cysts of *Giardia doudenalis* were measured, the average of cysts were 10-11 μm \times 8-9 μm , best detected in feces concentrated by sugar flotation method with specific gravity (1.18) (Fig. 2).

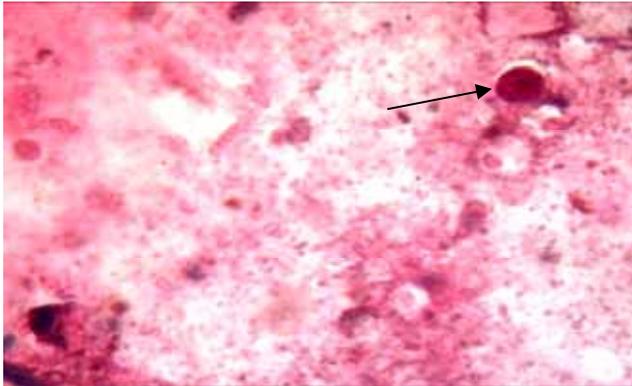


Fig. 1: *Cryptosporidium sp.*, oocyst by using modified Ziehl-Nelsen method (counter stain methylene blue). x1200.



Fig. 2: *Giardia doudenalis* cyst by using Giemsa stain. x1000.

Discussion

This is the first report of cryptosporidial infection in equines in Nineveh governorate. The infection was identified in 29 out of 107 animals from different regions in Nineveh.

Equine cryptosporidiosis 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 (16-19). In Iraq there are many studies which were conducted in different host such as calves, lambs, dogs, cats and rabbits with recorded different percentages of infection with *Cryptosporidium spp.* and *Giardia spp.* (3,5,20,21). While in equines we have no studies in our locality that deals with these parasites.

In this study the percentage of infection with *Cryptosporidium* was 27.10%, this result was agreement with other studies which were occurred in different countries such as Germany, Poland, Texas, Colorado, USA and Louisiana and these studies were recorded different percentage of infection ranged from 0.33_100%(22-29) according to the months of the year, there was a variation in the infection rate of *Cryptosporidium* (10-55.56%) and no significant differences between months of the year, these result were agreement with (3,30-32). The differences in the percentage of infection in the months may be related to different factors such as number of samples, environmental condition, age, sex, immunity status, stress factor...etc.

The age distribution among equines in this area covered by this study is relatively even, rang from one to 10 years old in our study over all rate of cryptosporidial infection. According to the age, infection was high in the 5-6 years age group which is higher than those reported by (33) who reported (3.2%) in horses ranging from one to 12 years only, and (13.9%) of horses aged $5 \geq$ years. In Morocco (34-36) few donkeys with old age over 12 years were infected, the reasons behind this are numerous such as type of life either feral or domestic, or immunity status, or in the sampling which is mostly taken from adult animals, or due to the unsuitable hygienic. Those infected horses with *Cryptosporidium* could be significant source of the parasite, both for other animals including human and for the environment, in addition horse faeces that used for soil fertilization also enhanced the like hood of contamination of food and water sheds.

In this study the percentage of infection with *Giardia doudenalis* was 19.63% this result was agreement with Kashash (20). Which recorded the infection rate in Baghdad was 14.6 % also this result was agreement with other studies occurred in different parts of the world (10,37), while (18) was recorded percentage of infection with *Giardia* 4.6%, 5% in horses respectively. According to the age high percentage of infection in age 2-4 years 5% in the same region.

While in racing horses two up to four years of age from two studies in the surrounding of Prague *Giardia* cysts were found in seven horses (35%) out of a total 20 animals (38, 39). Diaz (40) was reported high percentage of infection appears in several ages, while Kashash, Olsen, Wade

(20,29,31) showed that the infection of *Giardia* may occurred in all ages.

This is study reports for the first time the occurrence of *Giardia sp.*, in equines of the Nineveh.

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References

- Xiao L, Herd RP. Epidemiology of equine *Cryptosporidium* and *Giardia* infection. *Equine Vet J*.1994;26:1.
- Lecherallier MW, Norton WD, Leek G. *Giardia* and *cryptosporidium sp.*, in surface water. *App Environ Microbiol*.1991;57:2610-2616.
- Baker MH. The study of epidemiology experimental immunity and transmission for cryptosporidiosis in Nineveh governorate. [dissertation]: College of Veterinary Medicine, University of Mosul; 2005.
- Al-Bakray HS. Parasitic protozoa *Eimeria* and *Cryptosporidium* in goats Nineveh province. [master's thesis]: College of Veterinary Medicine, University of Mosul; 2002.
- Khalil, L.Y. Comparision of efficacy of diagnostic tests cryptosporidiosis in lambs and children in Nineveh governorate. [master's thesis]: College of Veterinary Medicine, University of Mosul; 2000.
- Fayer R, Speer CA, Dubey Jp. The general biology of *cryptosporidium*. In: fay er (ed): *Cryptosporidium* and cryptosporidiosis, 1-14 CRC press, Boca Raton.1997;ITP.
- Coleman Su, klei TR, French DD, Chapmann MR, Corstret RE. Prevalence of *Cryptosporidium* in equine in Louisiana. *Am J vet Res*. 1989;50:575-577.
- Da Voodi, Yand Noor M. The horse source of cryptosporidial infection for human.4th National congress of zoonoses in Iran.Tehran, Iran.2000;40.
- Olson ME, Thorlakson CL, Deselliers LM. TA. *Giardia* and *Cryptosporidium* in Canadian farm animals.*Vet parasites*.1997;68:375-381.
- Pavlassek I, Hessel, Stehlik I, Stikav. The first detection of *Giardia sp.* in horse in the Czech Republic. *Vet Med (praha)*. Mar.1996;40(3):81-6.
- WHO. Basic laboratory method in medical parasitology. Geneva, 1991;9-31.
- Henriksen SV, Pohlenz JFL. Staining of *Cryptosporidia* by a modified Ziehl_Nelsen technique. *Acta Vet Scand*.1981;22:594_569
- Ortega YR, Adam, RD. *Giardia*: over view and update. *Clin Inf Dis*. 1997;25:545.
- Deluol AM. Color atlas of parasitology. Format Utile, Varia, 1999: 34-63.
- Bruning LJ, Kintz BI. Computational hand book statistics. 2nd ed. Scott Foresman and Company England.1977;233-237.
- Reimeyer CR, Kline RC, Stauffer GD. Absence of *Cryptosporidium* oocysts in feces of neonatal foals. *Equine Vet J*.1984;16:217-218.
- Abou Eisha AM. *Cryptosporidium* infection in man and farm animals in Ismailia governorate. *Vet Med J Giza*.1994;42:107-108.
- Johnson E, Atwill ER, Filkins ME, Kalush J. The prevalence of shedding of *Cryptosporidium* and *Giardia sp.*, based on a single fecal sample collection from each of 91 horse used for back country reaction. *J Vet Diagen Invest*.1997;(9):56-60.
- Bray RE, Wickler SJ, Cogger EA, Atwill ER, London C, Gallino JL, Anderson TP. Endo parasite infection and *Cryptosporidium/ Giardia* in feral horses on public lands. *J Equine Vet Sci*.1998;18:41-43.
- Kshash KH, Mohammed TA. Clinical and Epidemiological study if Giardiasis in Cow in Baghdad. *Bas J Vet Res*.2006;5(1): 54-64.
- Suleiman EG. Diagnostic study of some intestinal protozoa in domestic rabbits in Mosul area. *Iraqi J Vet Med*.2005;19(2):123-133.
- Beelitz P, Göbel E, Gothe R. Artenspcktum und befallhä afigkeitvon endo- parasiten beifohlen und ihren Mutter stuten aus Zuchtbetrieben mit und ohne antihelmin-thika-prophylaxe in Oberbayern Tierär-ztt Prax.1996;(24):48-54.
- Cole DJ, Cohen ND, Snowden K, Smith R. Prevalence of and risk factor for fecal shedding of *Cryptosporidium parvum* oocysts in horse. *JAMA Vet Med Assoc*.1998;231,1296-1302.
- Forde KN, Swinke AM, Traub- Dargatz JL, Cheney JM. The prevalence of *Cryptosporidium/ Giardia* in trail horse population utilizing public lands in Colorado. *J Equine Vet Sci*.1998;18:38-40.
- Majewsk AC, Kasprzak W, Werner A. Prevalence of *Cryptosporidium* in mammals housed in Poznan zoologicd garden, Poland. *Acta Parasitol*.1997;42:195-198.
- Snyder SP, England JJ, Mcchesney AE. Cryptosporidiosis in immuno de ficient Arabian foals. *Vet Pathol*.1978;15:12-17.
- Coleman Su, klei TR, French DD, Chapman MR, Corstret RE. Prevalence of *Cryptosporidium sp.* In equids in Louisiana. *Am J Vet Res*.1989;50:575-577.
- Xiao L, Herd RP. Epidemiology of equine *Cryptosporidium* and *Giardia* in fections.*Equine Vet J*.1994;26:14-17.
- Olson ME, Thorlakosn CL, Desellier SL, Morck DW, Mc Alliste TA. *Giardia* and *Cryptosporidium* in Canadian farm animals. *Vet Parasitol*.1997;68:375-381.
- Lefay D, Naciri, M, Doirier P, Chemette R. Prevalence of *Cryptosporidium* in calves on France. *Vet Parasitol*.2000;89:1-9.
- Wade SE, Mohammed HO, Schaaf SL. Prevalence od *Giardia sp.*, *Cryptosporidium parvum* and *Cryptosporidium muris* (*C. adnersoni*) in 109 dairy herds in five countries of South Eastern. New York. *Vet Parasitol*.2000;93(1):1-11.
- Clavel A, Olivares JL, Fleta JC, Castillo J, Varea M, Ramos FJ, Arnal AC, Guilez J. Seasonality of cryptosporidiosis in children. *Eur J Clin Microbial Infect Dis*.1996;15:77-79.
- Tavassoli M, Sodagar_ Skandarabadi M, Soltanalineejad F. A survey on Cryptosporidial infection in horse in Urmia area, northwestern Iran. *Iranian J Vet Res*. 2007; 8: 86-90.
- Pearson RA, Russat M. Estimation of the liver weight and body condition of working donkey in Morocco. *Vet Rec*.1996;138:229-233.
- WallaeA. A assessing the efficacy of an antihelmtic programme on the health and welfare of working equines in Morocco. Research project for the society for the protection of animals a broad (SPANA) University of Liver pool.2003.
- Svendens ED. Work to improve the condition of donkeys and mules world wide. In :fielding, Dant P pearson, RA (eds), donkeys and mules and tropical agricultural development 9th eds. TVM Edinburgh.1991; 181-184.
- Johnson E, Atwill ER, Fikins ME, Kalush J. The prevalence of shedding of *Cryptosporidium* and *Giardia sp.*, based on a single fecal sample collection form each of 19 horses used for back country reaction. *J Vet Diagn Invest*.1997;9:56-60.
- De Souza DNB, TCB Bomfim, F Huber LCS Abboud, RS Gomes.Natural in fection by *Cryptosporidium sp. Giardia sp. Eimeria leukart* in three groups of equines different handlings in Rio De janerio, Brazil. *Vet Parasitol*.2009;160:3-4.
- Atwiller NK, Mc Dougald, Perea L. Cross sectional study of fecal shedding of *Giardia sp.* and *Cryptosporidium parvum* among packstocle in the Sierra Nevada Range, California USA.*Equine Vet J*. 2000;32:247-252.
- DiazV, Compous M, Lozano, Manas I and Gonzales J. Aspects of animal Giardiasis in Granada province (South Spain). *Vet Parasitol*. 1996;64(3):171-176.