



## Studies on Blood and Enteric Protozoans Infecting Camels At Behera Province, Egypt

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### Abstract:

This study was carried out to investigate the prevalence of blood and enteric protozoan parasites in freshly slaughtered camels at Behera Province, during the years of 2014 and 2015. 120 (66 male and 54 female) freshly slaughtered camels were examined, 94 (46 males and 48 females) (78.3%) were found to be infected with enteric protozoa, while 63 camels (24 males and 39 females) (52.5%) were found to be infected with blood protozoa. Concerning enteric protozoa, the highest rate was recorded for *Diplodinium* spp. (58%), followed by *Cryptosporidium parvum*. (24.16%), then *Eimeria* spp (13.33%). *Eimeria* spp. were *E.camili* (50%), *E. dromedarii* (31.25%) and, *E. pellerdyi* (18.75%). *Giardia intestinalis*, (5%) and *Balantidium coli* (4.16%). The prevalence of blood protozoa revealed that the highest rate was for *Theileria* spp., then *Anaplasma marginale* and *Trypanosoma evansi* 45.82, 15.8 and 1.66%, respectively. Regarding, seasonal dynamics of enteric protozoan parasites, *Cryptosporidium parvum*, *Eimeria* spp and *Diplodinium* spp showed their highest incidence during Winter, while *Balantidium coli* recorded the highest rate during Spring. Summer had the highest rate for *Theileria* spp. but there were abundance of *Anaplasma marginale* during spring and Autumn. Finally, *Trypanosoma evansi* were only found during winter and autumn.

**Key words:** Camel; blood protozoa; enteric protozoa; prevalence; Egypt

### INTRODUCTION

Camels are multipurpose animals in many places of the world because they are reared up for their meat, milk, hair and hides production, which could be as a source of additional income to nomadic herder. In Egypt, camels are mostly raised for meat consumption and other purposes, the imported camels mostly pass through Daraw quarantine at Aswan province. These camels come from different localities in Africa specially Sudan which has one of the largest populations of the one humped camels all over the world. Such importation may lead to the development and introduction of exotic diseases (Abd-Allah, 2007). Camels are susceptible to wide range of parasites that may cause many problems especially blood and gastrointestinal ones. The importance of blood parasites is not only as a direct cause of death but also affect the general health condition of camels *Theileria* spp is a tick-borne protozoan parasite belonging to the Phylum Apicomplexa (Boulter and Hall, 1999). Two species, namely *Theileria camelensis* and *dromedarii* have been reported from camel breeding areas of the world (Chhabra and Sangwan, 2006). Protozoal diseases particularly trypanosomosis, cause remarkable losses on animal production in all tropical and subtropical areas.

Trypanosomosis in camels is caused by *Trypanosoma evansi* and is transmitted from camel to camel by a number of species of haematophagous biting flies including *Tabanus*, *Stomoxys*, *Lyperosia* and *Haematobia* spp. (Stewart et al., 1996). *T. evansi* infection is a prevalent disease in camels (*Camelus dromedarius*), that causes considerable economic losses due to weakness, abortion in pregnant animals, and weight loss. In addition, the fatality rate resulted from trypanosomiasis may reach 100% in untreated camels (Derakhshanfar et al., 2010).

In Egypt, *T. evansi* in camels is enzootic and consider the fore-most impediment to dromedary camel productivity (Amer et al., 2011). Anaplasmosis, the tick-borne disease, is caused by an obligate intra erythrocytic rickettsial microorganism, *Anaplasma marginale* (*A. marginale*), of the order Rickettsiales, family anaplasmataceae. Coccidian parasites comprise of a large group of obligatory intracellular parasites (Duszynski et al., 1999). The coccidian genera, *Eimeria* and *Isospora* both infect camels, however only *Eimeria* species were recognized as the causing disease (Kaufmann 1996). Five reported *Eimeria* spp. have the capability to infect camels. They are intestinal parasites (Kaufmann, 1996 and Yakhchali and

**Cheraghi, 2007).** Cryptosporidiosis is one of the important zoonotic diseases caused by *Cryptosporidium spp.*; *Cryptosporidium (C.) muris*, *C. parvum* and *C. andersoni* are three species found in camelids (**Wang et al., 2008**). *Balantidium coli (B. coli)* is a ciliate protozoan and is frequently found in the intestinal tract of over 50 species of vertebrates, such as swine, human, non-human primates, and ruminants. In most cases, *B. coli* lives as a commensal organism in healthy human and animals. However, it is believed that under certain circumstances, *B. coli* may act as an opportunistic pathogen via the invasion of intestinal epithelium damaged by other infectious agents. Intestinal flagellates *Giardia intestinalis*, (*G. duodenalis*, or *G. lamblia*) infection occurs in a wide range of domestic and wild animals and also humans. *G. intestinalis* is a potential pathogen in livestock, causing diarrhea, weight loss, poor condition and lethargy (**Hunter and Thompson, 2005**). Also, *Diplodinium spp.* was the dominant in camel ciliated. **Selim et al. (1996)** stated the first description of *Diplodinium spp.* in Egypt detected in camel forestomach contents. **ORDER: Entodinomorphida, FAMILY: Ophr yoscolecidae, SUBFAMILY: Diplodiniinae, GENUS: Diplodinium**

**MATERIALS AND METHODS**

**Materials:**

The present study was conducted in Behera province. A total number of 120 camels of different ages (3 to 9 years) and sex were examined during 2014 and 2015. Blood specimens were collected from the ear veins for investigation of the blood protozoa. As well as, the detection of enteric protozoa has been done by collection of fresh fecal specimens and forestomach contents. These specimens were subjected to laboratory investigations for studying the prevalence of blood and enteric protozoal infection among camels.

**Methods:**

**Collection of blood and preparation of stained blood smears:** Specimens of 5 ml of blood from ear vein were

collected in screw capped tubes containing 0.5 ml of 1% Ethylene Diamine Tetra-Acetic acid (EDTA) solution and gently mixed. Thin blood smears were made, according to (**Adam et al., 1971**), stained by Giemsa's stain.

**Examination of enteric protozoa:** Concentration flotation technique using saturated salt solution was used according to (**Soulsby, 1982**), for detection of *Eimeria spp.* oocysts. The positive fecal samples were sporulated using 2.5% potassium dichromate solution for two weeks. Thin fecal smears were stained using Modified Zhiel-Neelsen staining technique (MZN) for the detection of *Cryptosporidium* oocyst according to (**Henriksen and Pohlenz, 1981**). Both blood and enteric specimens were microscopically examined according to the methods described by (**Higgins, 1986**).

**RESULTS**

The incidence of the identified parasites *Theileria spp.*, *Trypanosoma evansi*, *Anaplasma marginale*, *Cryptosporidium parvum*, *Eimeria spp.*, *Balantidium coli*, *Giardia intestinalis* and *Diplodinium spp.* were 45.82, 1.66, 15.8, 24.16, 13.33, 4.16, 5 and 58% respectively (Table 1).

**Blood parasites:** Out of 120 freshly slaughtered and examined camels 55 (45.82%) were positive for *Theileria spp.* Out of 55 detected cases, 30 males and 25 females were found to be positive, (Table 3). Seasonal profile of

Table 1: General incidence of blood and enteric protozoa in dromedary camels

	The examined specimens	Positive (+ve)	%
<b>Blood parasites</b>			
<i>Trypanosoma. evansi</i>	120	2	1.66
<i>Theileria spp.</i>	120	55	45.82
<i>Anaplasma marginale</i>	120	19	15.8
<b>Enteric protozoa</b>			
<i>Cryptosporidium parvum</i>	120	29	24.16
<i>Eimeria spp.</i>	120	16	13.33
<i>Balantidium coli</i>	120	5	4.16
<i>Giardia intestinalis</i>	120	6	5
<i>Diplodinium spp.</i>	120	70	58

Table 2: Seasonal prevalence of blood parasites infecting dromedary camels

Season	No. of the	<i>Theileria spp.</i>		<i>Anaplasma marginale</i>		<i>Trypanosoma evansi</i>	
		+ve	%	+ve	%	+ve	%
<b>Winter</b>	30	12	40	4	13.33	1	3.33
<b>Spring</b>	30	14	46.66	6	20	0	0
<b>Summer</b>	30	15	50	3	10	0	0
<b>Autumn</b>	30	14	46.66	6	20	1	3.33
<b>Total</b>	120	55	45.83	19	15.83	2	1.66

Table 3: General incidence of enteric protozoa in relation to sex

Parasites	No of positive			No of negative		
	Males +ve (%)	Female +ve (%)	Total (n=120) +ve (%)	Males	Females	Total
<i>Cryptosporidium parvum</i>	15 (51.7)	14 (48.3)	29 (24.2)	51	40	91
<i>Eimeria spp.</i>	9 (56.3)	7 (43.8)	16 (13.3)	57	47	104
<i>Balantidium coli</i>	2 (40.0)	3 (60.0)	5 (4.2)	64	51	115
<i>Giardia intestinalis</i>	2 (33.3)	4 (66.7)	6 (5.0)	64	50	114
<i>Diplodinium spp.</i>	35 (50.0)	35 (50.0)	70 (58.0)	31	19	50
<i>Trypanosoma. evansi</i>	1 (50.0)	1 (50.0)	2 (1.7)	65	53	118
<i>Theileria spp.</i>	30 (54.5)	25 (45.5)	55 (45.8)	36	29	65
<i>Anaplasma marginale</i>	10 (52.6)	9 (47.4)	19 (15.8)	56	45	101

the identified *Theileria* spp indicated that, the highest incidence in summer reached to 50%, followed by both spring and autumn 46.66%, then winter 40 %, (Table 2). Out of 120 freshly slaughtered and examined camels 2 (1.66%), were positive for *Trypanosoma evansi* Out of 2 detected cases, 1 male (50%) and 1 female (50%) were found to be positive. While, the seasonal dynamics of *Trypanosoma evansi* was in winter and autumn only 3.33% (Table 2). Out of 120 freshly slaughtered and examined camels 19 (15.8%) were positive for *Anaplasma marginale*. Out of 19 detected cases, 10 males (52.63%) and 9 females (47.36 %) were found to be positive, (Table 3) Seasonal incidence of *Anaplasma marginale* showed that, the highest prevalence was found in both spring and autumn 20%, followed by winter 13.33% then summer 10%, (Table 2, Fig. 1, 2, 3).

#### Enteric protozoa

***Cryptosporidium parvum*** : Out of 120 examined camel's fecal specimens 29 (24.16%) were found to be positive for *Cryptosporidium parvum* (Table 1). Out of 29 detected cases, 15 males (51.72%) and 14 females (48.27%) were found to be positive, (Table 3). Seasonal dynamics of the identified *Cryptosporidium. parvum* revealed that, the highest prevalence was found in winter 18 (60%),

followed by autumn 6 (20%), then spring 4 (13.3%) and summer 1 (3.33%), (Table 4, Fig. 4).

***Eimeria spp.***: Out of 120 examined camel's fecal specimens 16 (13.33%) were found to be positive for *Eimeria spp.*, (Table 1). Out of 16 cases, 9 male (56.25%) and 7 female (43.75%) were found to be positive, (Table 3). Seasonal dynamics of the identified *Eimeria spp.* indicated that, the highest prevalence was found in winter 11(36.66%) followed by autumn 5 (16.66%), no *Eimeria* spp. oocysts were detected during spring and summer, (Table 4). Three *Eimeria spp.* were identified, *E.pellerdyi* 8 (50%), *E.dromedarii* 5 (31.25%) and *E.cameli* 3 (18.75%). (Fig. 5).

***Blantidium coli***: Out of 120 examined camel's fecal specimens 5 (4.16%) were found to be positive for *Blantidium coli*, (Table 1). Out of 5 cases, 2 male (3.03%) and 3 female (5.55%) were found to be positive, (Table 4). Seasonal dynamics of the identified *Blantidium coli* indicated that, the highest prevalence was found in spring 2 (6.66%) and summer 2 (6.66%) and followed by autumn 1(3.33%), and no detection in winter 0 (0%), (Table 4, Fig. 6).

Table 4 Seasonal prevalence of enteric protozoa infecting dromedary camels

Seasons	No of the examined camels	<i>Cryptosporidium parvum</i>	<i>Eimeria spp.</i>	<i>Balantidium coli</i>	<i>Giardia intestinalis</i>	<i>Diplodinium spp.</i>
Winter	30	18 (60.0)	11 (36.7)	0 (0.0)	0 (0.0)	19 (63.3)
Spring	30	4 (13.3)	0 (0.0)	2 (6.7)	3 (10.0)	18 (60.0)
Summer	30	1 (3.3)	0 (0.0)	2 (6.7)	3 (10.0)	17 (56.7)
Autumn	30	6 (20.0)	5 (16.7)	1 (3.3)	0 (0.0)	16 (53.3)
Total	120	29 (24.2)	16 (13.3)	5 (4.2)	6 (5.0)	70 (58.3)

Data presented as number of +ve cases (%)

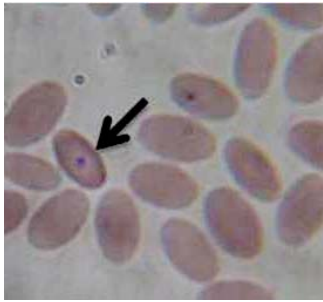


Figure 1: *Theileria* spp. (100x)

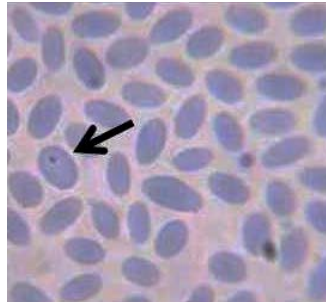


Figure 2: *Anaplasma marginale* (100x)

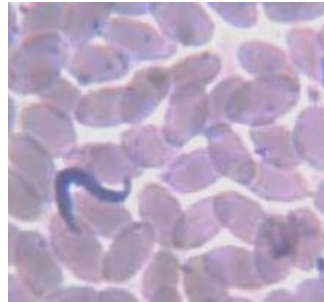


Figure 3: *Trypanosoma evansi* (100x)

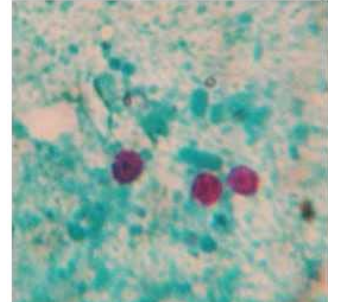


Figure 4: *Cryptosporidium parvum* (100x)

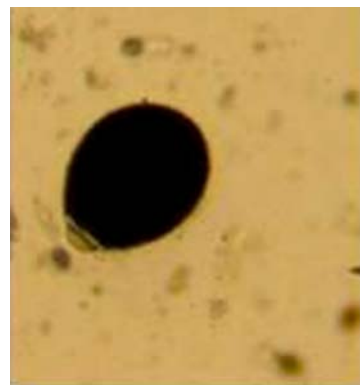


Figure 5: Oocysts of the three *Eimeria* spp. in the examined camel's fresh fecal specimens (40x)

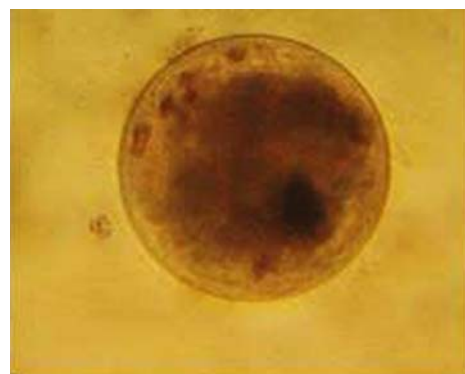
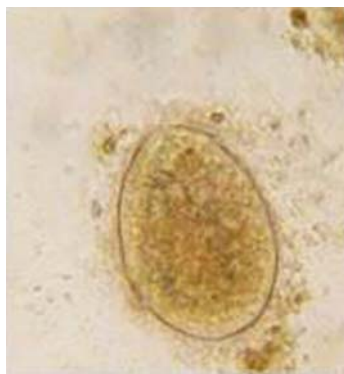


Figure 6: Trophozoite (Left) and cyst (Right) of *Balantidium coli* in the examined camel's fresh fecal specimens (40x)

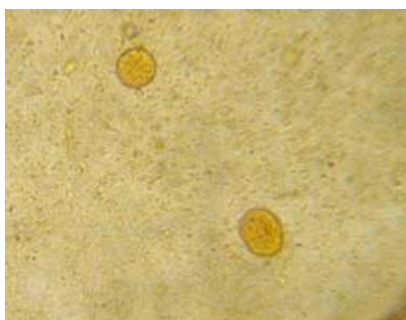


Figure 7: *Giardia intestinalis* in the examined camel's fresh fecal specimens (40x)



Figure 8: Trophozoite of *Diploidium* spp. in the examined camel's fresh fecal specimens (40x)

***Giardia intestinalis***: Out of 120 examined camel's fecal specimens 6 (5%) were found to be positive for *Giardia intestinalis*, (Table 1). Out of 6 cases, 2 male (33.33%) and 4 female (66.66%) were found to be positive, (Table 4). Seasonal dynamics of the identified *Giardia intestinalis*. indicated that, the highest prevalence was found in spring and summer 3 (10%) and no detection in both winter and autumn, (Table 4, Fig. 7).

***Diplodinium spp.*** :Out of 120 examined camel's fecal specimens 70 (5%) were found to be positive for *Diplodinium spp.*, (Table 1). Out of 70 cases, 35 male (50%) and 35 female (50%) were found to be positive, (Table 4). Seasonal dynamics of the identified *Diplodinium spp.* indicated that, the highest prevalence was found in winter 19 (63.33%), followed by spring 18 (60%), then summer 17 (56.66%) and autumn 16 (53.33%), (Table 4, Fig. 8).

## DISCUSSION

The present study was dedicated to investigate freshly examined slaughtered camels for the presence of blood and enteric protozoa. The study revealed that, the prevalence of *Trypanosoma evansi*, *Theileria spp.*, *Anaplasma marginale*, *Cryptosporidium parvum*, *Eimeria spp.*, *Blantidium coli*, *Giardia intestinalis* and *Diplodinium spp.* was 1.66, 45.82, 15.8, 24.16, 13.33, 4.16, 5% and 58% respectively.

Concerning, the prevalence of *Theileria spp.* infecting dromedary camels was 45.8% that somewhat similar to the results obtained by **El-Fayoumy et al. (2005)**, they detected the overall prevalence to be 44.8% . These results were lower than that obtained by **El-Refaii et al. (1998)**, who detected that, the overall prevalence was 62.1%. In contrast, these results was higher than that obtained by **Hamed et al. (2011)**, **Qalban et al. (2011)** and **Hekmatimoghaddam et al. (2012)**, they mentioned 6.75, 10 and 15.79% respectively. **Borji et al. (2009)** and **Sloboda et al. (2011)** found no positive camels in their survey. These variations in the different results may be attributed to different localities, population density of camels, environment, hygienic measures and camel management.

Seasonal profile of the identified *Theileria spp* indicated that the highest incidence in summer reached to 50% This result similar to that obtained by, **Mahrn (2004)** found that, Theileriosis was most prevalent during summer. This

might be due to correlation between the infestation and density of tick population.

Regarding, the prevalence of *Anaplasma marginale* infecting dromedary camels was 15.8%. This result disagreed with **Borji et al. (2009)**, who did not find *Anaplasma* organisms in their epidemiologic study, while as **Ismail et al. (2013)** detected only one positive case for *Anaplasma* organisms in their epidemiological study of 173 camels. Concerning, the prevalence of *Anaplasma marginale* among sex categories was 10 male and 9 female which revealed that, no significant difference between sex categories.

Regarding, the prevalence of *Trypanosoma evansi* infecting dromedary camels was 1.66% relatively similar result obtained by **Laila et al. (2001)** and **Abd-Elmaleck et al. (2014)**, was 4.1% and 3.06%,. On other hand, our result lower than that obtained by **Mahrn (2004)** (11.6%) and **Baraka et al. (2005)** (26.6%). Such variations in prevalence of blood parasites may ascribe to several reasons, including different localities, population density of camels, environment and hygienic measures. Concerning the prevalence of *Trypanosoma evansi* among sex categories was 50% male and 50% female which revealed that, no significant difference between sex categories. Our result were similar to **Bogale et al. (2012)** who, detected that no statistically significant difference. Regarding the seasonal prevalence of *Trypanosoma evansi*. infecting camels in the present study, we found *Trypanosoma evansi* only in winter and autumn 3.33%. This result nearly similar to that obtained by **Zayed et al. (2010)** who detected that, the highest prevalence rate was observed in winter 20% followed by summer 10%, spring 2.08% and 0% in autumn. The seasonal variation in prevalence of blood protozoa may be attributed to the different environmental changes; temperature, humidity and rainy or dry season, animal factors, farm management other stress factors and Tabanidae which considered the main mechanical vectors of *T. evansi*.

The current study showed that the incidence rate of *Cryptosporidium parvum*. infecting dromedary camels was 24.16% that similar to some extent to the results obtained by **Nazifi et al. (2009)**, **Sazmand et al. (2012b)** and **Abdel-Wahab and Abdel-Maogood, 2011**, they detected the overall prevalence to be 19.9, 20.33 and 19.3% respectively. In contrary our current results were lower than that obtained by **Razawi et al. (2009)**. As well as, our

results was higher than that obtained by **Borji et al. (2009)**, **Saleh and Mahran (2007)** and **Yakhchali and Moradi (2012)**, they detected the overall prevalence to be 1.96, 3.37 and 10% respectively. These variations could be due to the difference in the environmental condition and hygienic measures. Concerning the seasonal prevalence of *Cryptosporidium parvum*. in the current study, the highest prevalence rate was detected to be 60% in winter followed by autumn 20%, spring 13.33% then summer 3.33%. This result was nearly similar to the result that, obtained by **Mohamed (2013)** in Egypt, who detected that, the seasonal variation of *Cryptosporidium spp.* was higher in spring 13.33%, followed by winter 10.48%, autumn 7.62% then summer 4.76%. Concerning, the prevalence of *Cryptosporidium spp.* among sex categories was 12.5% male and 11.6% female which revealed that, no significant difference between sex categories. Our result were nearly similar to **Sazmand et al. (2012b)** whose, Statistical analysis showed no significant relation between infection and sex.

*Eimeria spp.* infecting dromedary camels, the results showed that the incidence rate 13.33% that similar to the results obtained by **Yakhchalim and Cheraghie (2007)**, **El-Salahy et al. (2000)**, **Kawasmeh and El-Bihari (1983)**, **Abubakr et al. (2000)**, **Harfoush and Abd El-Aal (2007)** and **Yakhchali and Athari (2010)**, they detected the overall prevalence to be 12.8, 13.3, 14, 15.3, 18.7 and 20.73% respectively. Vice versa , the current results were lower than that obtained by **Mohamed (2013)**, **Wahib and Magda (2002)**, **El-Metenawy (1998)**, **Rangarao and Sharma (1997)**, **Kasim et al. (1985)** and **Biu et al. (2003)**, they detected that the overall prevalence to be 24.29, 31.4,34,40 , 41.6 and 48.9% respectively. As well as, our results were higher than that obtained by **Mahran (2006)** and **Sazmand et al. (2012a)**; they detected the overall prevalence to be 10.94 and 9.51% respectively.

Concerning the seasonal prevalence of *Eimeria spp.* in the current study, the highest prevalence rate was detected to be 36.66% in winter followed by autumn 16.66% and 0% in both spring and summer. This result was nearly similar to the result that, obtained by **Borji et al. (2009)** in Iran who, detected that, the seasonal prevalence of infection with *Eimeria spp.* of camels was 38.29% in winter followed by autumn 17.39%, summer 15.62% and spring 11.23%. Concerning, the prevalence of *Eimeria spp.* among sex categories was 7.5% male and 5.83% female. The most prevalent *Eimeria* species in this work was *E.*

*cameli*, followed by *E. dromedarii* then, *E. pellerdyi*. Similar results were obtained by **Mahran (2006)** in Egypt.

Concerning the prevalence of *Blantidium coli* infecting dromedary camels was 4.16% which agreed to **Wahba and Radwan (2009)**, **El-Tayar et al. (2012)** and **Har foush and Abd El-Aal (2007)**, who detected that the incidence rate of 4.76% in Cairo, 3.3% and 3.73% in Matrouh respectively. In addition, our results were lower than that obtained by **Rewatker et al. (2009)**, **Partani et al. (1998)** and **Tekle and Abebe (2001)**, who detected prevalence rate of 7.14, 30.2 and 11.92% respectively. There are a few reports about the presence of *B. coli* in camel fecal samples. Among sex categories females was higher than males. This might be attributed to stress of pregnancy, lactation and hormonal factors. The reservoir host for *B. coli* in Islamic countries is unknown. Camel has been proposed as a reservoir host for *B. coli* in Islamic countries, however, there are few documents.

The incidence rate of *Giardia spp.* in this study was 5% while **Beck et al. (2011)** confirmed the absence of *Giardia spp.* in the captive camels in zoo of Croatia this in contrast with **Radhy et al. (2013)**, who stated incidence rate of 100%. Concerning the prevalence of *Diplodinium spp.*, infecting dromedary camels was (5%) limited number of studied have been reported. Among sex categories females was higher than males. This might be attributed to stress of pregnancy, lactation and hormonal factors. These differences in enteric protozoans could be attributed to difference in environment condition between the countries besides the difference in the number of camels included in these studies

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