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SOME PARASITIC CAUSES OF DIARRHEA IN CALVES AT BEHERA GOVERNORATE

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ABSTRACT

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Three hundered faecal samples from diarrheic calves were examined using concentration flotation technique using saturated salt solution to detect *Eimeria* Spp.Oocysts and using Sheathers solution to detect *Giardia* species, Modified Ziehl-Neelsen staining technique to detect *Cryptosporidium parvum*. The results indicated that 158 (52.66%) were postive for protozoal parasites as *Eimeria* species (27%), *Cryptosporidium parvum* (14.66%) and *Giardia* species (11%). Parasitological examination revealed that isolated five species of *Eimeria* in calves were *E.bovis, E.zuernii, E.subspherica, E..cylindrica* and *E.ellipsiodalls. E. bovis* and *E.zuernii* (27.1%) and (23.4%) were the most prevalent species infecting calves followed by *E.subspherica* (14.8%), then *E.ellipsoidalis* (13.5%) finally *E. cylindrical* (9.8%). *Eimeria* Oocysts were demonstrated in all age groups of animals. However, it was evident that the prevalence of the disease was significantly higher in 16-20 weeks old (46.6%). While *Cryptosporidium parvum* Oocysts was found to be decreased with increasing age of calves. It was found the most affected age group was between 1-4 weeks old (32%) and Cysts of *Giardia* spp. were found to be higher at age group 4–8 weeks old (30%). Seasonality over all prevalence of *Eimeria* spp., *Cryptosporidium parvum* and *Giardia* spp. was higher in samples taken during winter than samples taken during summer.

Key words: *Eimeria spp., cryptosporidium, diarrhea in calves.*

INTRODUTION

The most important cause of calf morbidity and mortalilty is diarrhea (Heat, 1992), Neonatal calf diarrhea continues to be the 1st cause of calf mortality in Egypt which ranges between 27.4-55% of the total death in young calves (Ahmed, 1980).

Cryptosporidium, Giardia and *Eimeria* species are among the most common and important enteropathogens in calves (Maddox – Hyttel *et al.*, 2006; Bartles *et al.*, 2010). The most important clinical sign they may cause is watery diarrhoea, occasionally contaning blood in *Eimeria* infection. Other less obvious signs are fatigue, fever and reduced appetite (Fitzgerald, 1980). High infection rate, and lack of preventive and therapeutic measures when clinical disease manifests, can lead to out breaks and high mortality among calves. Moreover, the sub clincial effects of infection, which often result in recovery but lower weight gain, are also important to animal health and productivity (Daugschies *et al.*, 1986). Bovine coccidiosis has a world wide distribution and result in economical losses. More than twelve different species of *Eimeria* in cattle and buffalo have been documented up till now (Abebe *et al.*, 2008).

Most commonly pathogenic species are *E. bovis* and *E.zuernii* causing mortality and morbidity by disturbing absorption mechanisms (Cicek *et al.*, 2007).

Coccidiosis in cattle is observed in all age groups but it is most common and important in young animals, in associations with other entropathogens coccidia have been indicated as an important cause of diarrhea in calves. *Cryptosporidium parvum*, first reported as entero pathogen in a calf (Panciera *et al.*, 1971), has now been reported as prevalent and wide spread in calves from 4 days to 4 weeks of age by numerous investigators in many countries. Clinical neonatal bovine Cryptosporidiosis is usually a result of infection with *C. parvum*. *Cryptosporidium* has direct life cycle which is completed in 3 days and transmission is fecal – oral. Cryptosporidiosis is considered to be zoonotic disease (Miron, 1991) and the most common route of infection in close contact with diarrheic feces. Due to the limited availability of effective drugs the control of Cryptosporidiosis relies mainly on hygienic measures and good management (De Graaf *et al.*, 1999).

Giardia intestinalis (synomyms *G. duodenalis* and *G. lamblia*) is globally distributed and is a common protozoon parasite in many species of animals and man. The public health implication of Giardiasis are well known and it's effect on calf performance is not conclusive. The earliest reports of *Giardia* were in India and Egypt were made by (Deshpande and

Shostri, 1981) and (Suvillan *et al.*, 1988)

There is a close association between the prevalence of the *Cryptosporidium* and *Giardia* infection and age of animal (Lassen, 2011).

The aim of this study was to:

1- Estimation the prevalence, age and seasonal distribution of *Cryptosporidium*, *Giardia* and *Eimeria* infection among calves.

2- Identification of various species of *Cryptosporidium*, *Giardia* and *Eimeria* affecting calves.

MATERIALS and METHODS

1- **Animals**: a total number of 300 fecal samples from cow calves from May 2011 to April 2012 were collected from different localities in Behera Governorate. Calves aged from 1 week to 20 weeks.

2- **Samples**: faecal samples were collected directly from the rectum using plastic hand gloves, each glove being marked to indicate animals number age and date of sampling.

Parasitological Examination.

1- Concentration flotation technique: according to Soulsby (1982), using saturated salt solution for detction of *Eimeria spp. oocysts* and Sheathers solution for detction of *Giardia* spp.cysts.

2- Sporultion of *Eimeria species* oocysts: according to Soulsby (1982) the positive fecal samples were sporulated using 2.5% potassium dichromate solution for one week.

3- Modified Ziehl- Neelsen staining technique (MZN): was used for diagnosis of *Cryptosporidium parvum* oocyst according to Henriksen and Pohlenz (1981). Thin fecal smears were prepared on glass slides, dried at room temp., fixed in methanol 96% for 2-5 min., then fixed in flame samples were stained in concentrated carbol fuchsin for 20-30 min.The discoloration procedure was realized with 3% hydrochloric acid for 20-60 seconds, then smears were washed with running water and counter stained with solution of malachite green for 3- 5 min. rinsed in tap water and air dried.

4- Cysts of *Giardia* were found by floating the sample in Sheathers solution which has been found superior in getting *Giardia* Cysts to float, dropwise application of dilute Lugol's iodine solution (10.5%) to facilitate microscopic examination of *Giardia* according to Levine (1985).

5- Smears were examined under light microscope with x40 and x 100 magnifications, Non sporulated oocysts, sporulated oocysts and cysts size were measured using bright field microscopy with a calibrated eye piece micrometer.

6- Identification of different oocysts & Cysts, according to Levine (1985).

RESULTS

Prevalence of *Cryptosporidium* oocyst, *Giardia* cyst. and *Eimeria* oocysts:

As shown in Table (1) out of the examined 300 faecal samples from diarrheic calves 158 (52.66%) were positive for oocysts of total *Eimeria* spp., total *Cryptosporidium parvum* and total Cysts of *Giardia* spp. 27%, 14.66% and 11%. Table (2) Parasitological examination revealed that isolated five species of *Eimeria* in calves were *E.bovis, E.zuernii, E.subspherica, E.cylindrica and E.ellipsoidalis.* The most prevalent species infecting calves were *E.bovis and E.zuernii* (27.1%), (23.4%) followed by *E.subspherica (14.8%) and E.ellipsoidalis* (13.5%) respectively. The lowest prevalent was *E.cylindrica* (9.8%). Mixed infection involved two or three *Eimeria* species was observed in 11.11% of the samples.

Table (3) *Eimeria* oocysts were demonstrated in all age groups of animals. However, it was evident that the prevalence of the disease was significantly higher at 16 - 20 ws (46.6%). While *Cryptosporidium parvum* appeared to be inversely related to age, where the prevalence of oocysts was found to be decreased with increasing age of calves. It was found the most affected age group was between 1-4 ws (32%). Cysts of *Giardia* spp. were found high at age group 4–8 ws (30%). Table (4) for seasonal variations reveal that over all prevalence of *Eimeria* spp., *Cryptosporidium parvum* and *Giardia* cysts, was higher in samples taken during winter than samples taken during summer.

Morphology of *Cryptosporidium* oocyst, *Giardia* cyst.and *Eimeria* oocysts:

Fig. (1) Microscopical examination of Acid-fast stained smears revealed ovoid oocysts of *Cryptosporidium*, measured of 4–6 um in diameter. The oocysts were spherical to ovoid in shape stained red to pink with granular appearance against green back ground.

Fig (2). Microscopical examination of iodine stained smears revealed *Giardia* cyst is oval cyst measured 7–11 um long and 7–9 um wid and a 0.3 um thick outer wall of cystic wall internally there are four nuclei. Figs (3-7) represent non sporulated and sporulated oocysts of *Eimeria* spp.

Table 1: Incidence of Protozoan Parasites Infect Calves at Behera Governorate.

No. Of Ex. calves	Single *Eimeria spp.*		Single *Crypto. Spp.*		Single *Giardia Spp.*,		Mixed infection	Total *Eimeria*		Total *Crypto.*		Toal *Giardia*		Total infec. Calves	
	No	%	No	%	No	%	*Eimeria + Crypto (2)*	No	%	No	%	No	%	No	%
300	75	25	39	13	27	9	*Eimeria + Giardia (3)* *Eimeria + Giardia + Crypto (1)* *Giardia + Crypto (2)*	81	27	44	14.66	33	11	158	52.66

Table 2: Prevalence and Characters of Different *Eimeria spp.* Isolated from Calves

Eimeria spp.	No.	%	Shape	Size um	Micropyle	Sporulation Time days
E.bovis	22	27.1	Ovoid	27x 20.3	Present	2 – 3
E.zuernii	19	23.4	Spherical	17.8x 15.6	No	2 – 3
E.subspherica	12	14.8	Round or Subspherical	11 x 10.4	No	4 – 5
E. cylindrica	8	9.8	Cylindrical	15 x 12	No	2 – 3
E.ellipsoidalls	11	13.5	Ellipsiod to slightovoid	12 x 8	No	2 – 3
Mixed in fection	9	11.11				

Table 3: Prevalence of Different Protozoan Infection in Relation to Age of Calves

Age	No. of exam calves	*Eimeria*		*Cryptosporidia*		*Giardia*	
		No.	%	No.	%	No.	%
1 – 4 ws	50	7	14	16	32	3	6
4 – 8 ws	50	8	16	14	28	15	30
8 – 12 ws	75	26	34.6	8	10.6	6	8
12 – 16 ws	50	5	6.1	5	10	5	10
16 – 20 ws	75	35	46.6	1	1.3	4	9
Total	300	81	27	44	14.66	33	11

Table 4: Seasonal Variations of Prevalence of Different Protozoal Infection

Seasons	No. of exam calves	*Eimeria*		*Crypto.*		*Giardia*	
		No.	%	No.	%	No.	%
Spring	75	12	16	8	10.6	3	4
Summer	75	10	13.3	4	5.3	4	5.3
Autumn	75	25	33.3	13	17.3	11	14.6
Winter	75	34	45.3	19	25.3	15	20

Fig (1)

Fig (2)

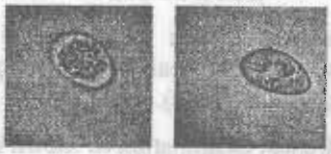

Fig (3)

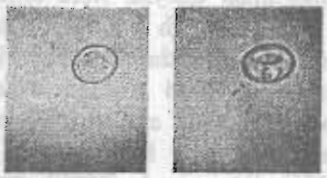

Fig (4)

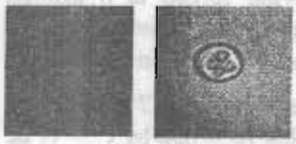

Fig (5)

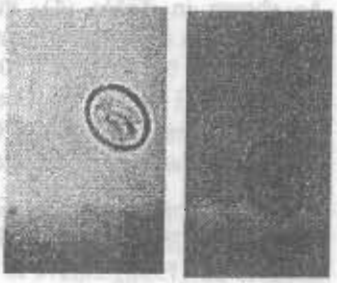

Fig (6)

Fig (7)

Fig (1) Oocysts of *Cryptosporidium parvum* stained with Modified Ziehl-Neelsen stain X100

Fig (2) Cysts of *Giardia spp.*stained with iodine stain X40

Fig (3) Non sporulated and sporulated oocysts of *E.bovis* X40

Fig (4) Non sporulated and sporulated oocysts of *E.zuernii* X40

Fig (5) Non sporulated and sporulated oocysts of *E.subspherica* X40

Fig (6) Non sporulated and sporulated oocysts of *E.cylindrica* X40

Fig (7) Sporulated oocysts of *E.Ellipsoidalis* X40

DISCUSSION

Cryptosporidium, Giardia and *Eimeria* infections have been reported from calves in many parts of the world (Hamnes *et al.*, 2006).

The parasitological examination as shown in Table (1) revealed that 158 (52.66 %) of calves examined were infected with one or more parasitic protozoan agents. The result of this study confirmed the fact that, protozoal infection were wide spread in diarrhoeic calves, this is in accordance with previous studies (Goz. *et al.*, 2006 and Gul *et al.*, 2008) in Turkey.

Eimeria oocysts were identified in (27 %) calves, these results are nearly to that recorded by Gul *et al.* (2008) 22.53% in Turkey. Over all prevalence of *Eimeria* in present study is lower than perivously reported by Gasmir *et al.* (2011) 47.59% in Sudan and Yu *et al.* (2011) 34. 94% in China while our results was higher than this reported by El. Sheriff *et al.* (2000) 12 . 05% in Egypt. This variation is most likely attributed to the differences in management and hasbandry practice of the study animals in different countries. Table (2) Five *Eimeria species* were identified in calves in this study namely *E. bovis, E.Zuernii, E. Subspheica, E. cylindrica and E. ellipsoidalis*, similar number of species has been described in surveys performed by El Sheriff *et al.* (2000) and Mary (2003).

Table (2) demonstrared that the prevalence of *E. bovis* and *E zuernii*, (27.1%) and (23.4%) which are most prevalent species for calves this concurs with the findings of Harfoush *et al.* (2005), Cicek *et al.* (2007), Abebe *et al.* (2008) and lassen *et al.* (2011).

Table (4) The prevalence of *Eimeria* spp. oocysts were highest in the age group of the 16-20 weeks old, this results were in accordance with the finding of Cicek *et al.* (2007); Abebe *et al.* (2008) and Priti *et al.* (2008), this data is in contrast with Sanchez *et al.* (2008) who found high percentage of infection 21–45 day.

Seasonal prevalence of coccidiosis among calves was higher in Winter (45.3%) Followed by Autumn (33.3%), Spring (16%) and Summer (13.3%) Table (4). Similar results were recorded by El Sheriff *et al.* (2000) and Mary (2003) in Egypt who reported higher prevalence during month of rain, while Sanchez *et al.* (2008) found that highest incidence in spring and autumn than winter and summer. This difference may be due to the different localities, seasonal and climate variation.

Cryptosporidium and *Giardia* infections have been reported for cattle in many parts of the world, prevalence data have often varied markedly. The high prevalence of *Cryptosporidium* and *Giardia* in neonates and young calves is well known and unambiguously age dependent (Xiao and Herd 2004). The findings of the present study showed of *Cryptosporidium.*oocysts were identified in (14.66%) in neonat calves by Modified Ziehl Neelsen staining technique, these results were relatively Similar to the values recorded by EL-Shemy, (2006), El.Khodery and Osman (2008), Amal and Reda (2009) and El Kelesh *et al.* (2009) (14%,14.19%, 14% and 14%). While our result is relatively lower than the values recorded by, El sherif *et al.* (2000) 21.92 % and Majeed *et al.* (2011) 38.8% Table (1). On other hand our result was higher that obtined by Cokline *et al.* (2009) 6.2%. These differences might be attributed to the difference in localities, breeding and management system. *Cryptosporidium* oocysts were found in all age categories but with high prevalence in 1-4ws (35%), these results revealed that Cryptosporidios is a disease of young ages and gradual decrease of infection rate occuared with age progression, these finding were emphasized by Bartels *et al.* (2010). Mean while, O'Handley *et al.* (1999) stated that *Cryptosporidium* was important pathogen when calves were less than one month, also EL Sherif *et al.* (2000); El. Dessouky and Nabila (2005); Amal and Reda (2009) and Majeed *et al.* (2011) said that calves less than one month recorded the highest degree of infection.

As shown in Table (4), the highest incidence of infection was recorded in winter (25.3%), followed by spring (17.3 %), autumn (10.8 %) and summer 8%, Bendali *et al.* (1999) and Lefay *et al.* (2001) found that the highest incidence of Cryptosporidiosis was in winter and lower infection rate was in summer.

From the presnt study *Giardia* spp.cysts were identified in (9%) of calves, these result was similar to Iburg *et al.* (1996) 10.4% in Denmark and Gul *et al.* (2008) 9.3%, While higher result observed by, Ruest *et al.* (1998) 26.6% in swedan, and Olson *et al.* (2004) 73 % British Columbia, While lower result observed by Xiao (1994) 5.6% These difference may be due to the different localities , seasonal and climate variation Table (1).

This highest rate of prevalence of *Giardia* in the age group of the 4-8 weeks old these result similar to Davoudi *et al.* (2011) who found the highest dgree of infection in 8 weeks old 54.38% Table (3). Seasonal prevalence of *Giardia* among calves highest rate of incidence was detected in winter season (20%) this result agree with Misic *et al.* (2006) and this result disagree with Davoudi *et al.* (2011) who found the highest degree of incidence was in summer(37.6%) and least incidence was in winter (16.8%) These differences might be attributed to the difference in localities, breeding and management system Table (4).

CONCLUSION

This study reveals that *cryptosporidium* spp., *Giardia* spp., and *Eimeria* spp., are the most Frequent parasites in calves at Behera Governorate These infections play an important role in animal production as it has been demonstrating that these parasites are able to cause diarrhea which negatively inflnuce growth impair feed conversion and reduce milk production.

Giardia and *Cryptosporidium* may also act as an infection source for humans by direct contact with contaminated faces or water.

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بعض الاسباب الطفيلية للاسهال في العجول بمحافظة البحيرة

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اجريت هذه الدراسة على ٣٠٠ عينة من البراز من العجول البقرى المصابة بالأ سهال تراوحت أعمارها من أسبوع حتى ٢٠ أسبوع فى محافظة البحيرة خلال الفترة من مايو الى٢٠١١أبريل٢٠١٢ للوقوف على نسبة الاصابة بالأوليات وكانت نسبة الأصابة الكلية ٥٢,٦٦%. بينما حويصلات الأيميريا وحويصلات الكربتوسبوريديا والجياردیا (٢٧ % و ١٤,٦٦% و١١%) وذلك باستخدام طريقة التعويم باستخدام محلول ملح مشبع لعزل حويصلات الأيميريا و باستخدام محلول سكر مشبع لعزل حويصلات الجيارديا وصبغة الزيل نيلسون المحورة لحويصلات الكربتوسبوريديا وتم تصنيف خمسة أنواع من الحويصلات البوغية لايميريا العجول وهى ايميريا (بوفس -زيرنى- صيبسفريكا - سيلندريكا- البيسويدالس) باستخدام ٢.٥%محلول البوتاسيوم داى كرومات وقد وجد ان اكثر الأنواع شيوعا هما ايميريا (بوفس –زيرنى) بنسبة ٢٧,١% و٣,٤ ٢% يليها صيبسفريكا بنسبة١٤,٨%ثم البيسويدالس بنسبة١٣,٥% ، أخيرا سيلندريكا بنسبة٩,٨% . حويصلات الأيميريا وجدت فى كل الفئات العمرية للحيوانات وكان معدل الأصابة أعلى فى الفئة العمرية من (١٦- ٢٠) اسبوع بنسبة ٤٦, ٦ %٠بينما حويصلات الكربتوسبوريديا لوحظ انها تقل كلما زاد العمر وكان معدل الأصابة أعلى فى الفئة العمرية من (١- ٤) اسبوع بنسبة ٣٢ %٠ وأخيرا حويصلات الجياردیا أعلى فى الفئة العمرية من(٤- ٨) اسبوع بنسبة ٣٠ %٠ وان معدل الاصابة (وحويصلات الأيميريا وحويصلات الكربتوسبوريديا و الجياردیا) كان أعلى فى فصل الشتاء مقارنة ببقية فصول السنة٠