



THE INCIDENCE OF CRYPTOSPORIDIUM INFECTION AMONG FRIESIAN AND BUFFALO CALVES IN MINUFIYA GOVERNORATE

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ABSTRACT

Parasitic diseases constitute one of the important problems affecting the general condition of farm animals causing great losses in the animal productivity or may lead to their death. The present study intended to estimate the prevalence of Cryptosporidium infection in dairy calves of different ages (1-3 months). It aimed later to determine relation between incidence rate and the age of calves. A total of 717 fecal samples Friesian and buffalo calves were selected from Minufiya governorate. These samples were examined using the safranin staining method. The highest prevalence of Cryptosporidium (56.32%) was observed in one-week-old calves. The age specific variation for the occurrence of parasites was statistically not significantly different for Cryptosporidium. The highest rate of infection by Cryptosporidium was recorded in summer (46.39%) then spring (45.65%), autumn (24.18%). Finally winter (16.33%) The highest rate of infection by Cryptosporidium was recorded in August (60.00%), Mar (54.56%) and Apr (50.00%). While in December, the incidence was (23.08%). Moreover, the statistical analysis showed that the cryptosporidium oocyst shedding among diarrheic calves was significantly higher ($p < 0.001$) than that of non-diarrheic calves. While in buffalo calves, the highest prevalence of Cryptosporidium (34.07%) was observed in one-week-old calves. The age specific variation for the occurrence of parasites was statistically not significantly different for Cryptosporidium. The highest rate of infection by Cryptosporidium was recorded in summer (34.19%) then spring (18.18%), autumn (14.29%). Finally winter (8.70%) The highest rate of infection by Cryptosporidium was recorded in August (57.14%), May (40%) and June (31.68%). While in December, the incidence was 10.00%. Moreover, the statistical analysis showed that the cryptosporidium oocyst shedding among diarrheic calves was significantly higher ($p < 0.001$) than that of non-diarrheic calves.

Keywords: Calves, Cryptosporidium, dehydration, diarrhea, enteritis, prevalence.

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1. INTRODUCTION

Cryptosporidiosis is a major problem in livestock production in Egypt and throughout the world (Ibrahim, 2007). Gastroenteritis in newborn calves causes, diarrhea, enteritis dehydration, and high morbidity and mortality, leading to significant economic losses in Egypt

(Ashraf, 2007). Neonatal diarrhea is a major source of economic loss in the cattle industry and a leading cause of calf mortality in most countries. Calf scour is not a single disease entity; it is a clinical syndrome associated with several diseases characterized by diarrhea. Regardless of the cause, absorption of fluids from the intestine is altered, leading to life-threatening electrolyte imbalances.

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The scouring calf loses fluids, rapidly dehydrated, and suffers from electrolyte loss and acidosis. Infectious agents may cause initial damage to the intestine, but death from scours usually results from dehydration, acidosis, and loss of electrolytes. Identification of infectious agents that cause scours is essential for implementation of effective preventive and treatment measures (Radostits et al., 2007). The main causes are bacterial infections, viral and protozoan agents can infect the animals (Diaz-Lee et al., 2011). The most common protozoa agents responsible for the diarrhea are *Cryptosporidium*, *Eimeria* and *Giardia* (Göz et al., (2006). The presence of the *cryptosporidium* along with enteropathogenic *Escherichia coli* may increase the mortality rate in calves affected with diarrhea (Shokier, 1999). This study was directed to determine the incidence, seasonal dynamics, and the age susceptibility of *cryptosporidium* among calves in Minufiya governorate.

2. MATERIALS AND METHODS

2.1. Animals:

This study was carried out during the period from December 2011 to November 2012. It was conducted on 371 Friesian calves and 346 buffaloes calves from different localities at *Minufiya Governorate Berkat EL Sabh and Shiben EL-kom*. (Table I and Table 3). These calves were fed on the whole milk and were exposed to full parasitological and clinical examinations. The examined calves were classified into 4 groups as follows: Based on age of calves.

Group 1: This group consisted of 261 Friesian calves of less than one month old.

Group 2: This group consisted of 110 Friesian calves from one month to less than 3 months.

Group 3: This group consisted of 260 buffalo calves of less than one month old. Group

4: This group consisted of 86 buffalo calves aged from one month to less than 3 months. These groups were sub grouped according to the consistency of the feces as follows: Non diarrheic calves: 134 Friesian calves and 120 buffalo calves.

2.2. Faecal samples:

Diarrheic calves: 237 Friesian calves and 226 buffalo calves. Fecal samples were collected from Friesian diseased and treated (48 hours P.T.) post treated calves and buffalo calves, suffered clinically from diarrhea and non-diarrhea calves. Macroscopically and microscopically examination were applied on each fecal sample. The feces were filtered through two layers of gauze to remove the coarse particles and stored in an equal amount of 2.5% potassium dichromate solution at 4°C for storage till 6 months the time of examination microscopically (Khalil, 1993).

2.3. Parasitological examination of the faecal samples:

2.3.1. Macroscopic examination:

It was applied to detect the abnormalities in consistency, color of the feces and presence of other abnormalities.

2.3.2. Microscopic examination:

The fecal samples were examined by ordinary direct smear method and sedimentation by using saline solution smear method. These methods were performed according to Belding (1952).

2.3.3. Staining procedures:

The fecal samples were subjected to staining technique by safranin- methylene blue. This method was performed according to Baxby et al., (1984). Fecal smears were prepared from the examined samples. After air drying, the fecal films were fixed briefly by one pass through the Bunsen flame. Then, the films were fixed with 3% HCl in 100% methanol for 3-5 minutes and then washed with tap water. Smears were stained with 1% aqueous

red safranin for 60 seconds. Heat thoroughly preferably until boiling occurs with adding more stain and continue the heating if necessary. Then, smears were washed with water. Counter-staining was carried out with 1% methylene blue for 30 seconds. Then smears were washed with water, blotted, and dried.

2.3.4. Oocyst separation smear:

This method was performed for examination and measurement of the oocyst according to Kilani and Sekla (1987). The modified dichromate sucrose solution was placed in a 50 ml screw-cap centrifuge tube, inverted 10 times and centrifuged at 800 r.p.m for 10 minutes. then the surface layer was washed with 0.85% saline and centrifuged at 1200 r.p.m for five minutes. then washing was repeated three times. then the sediment was resuspended in saline for animal inoculation and was kept at 4°C until use.

2.4. Statistical analysis:

It was done according to SAS (1987), T-test and Chi 2 test to show the relations between *C. Parvum* infections and months ages and case of fecal.

3. RESULTS

3.1. Clinical investigation and Macroscopic examination:

Diseased calves showed diarrhea in the form of watery feces of whitish or yellowish in color and some samples contained blood and mucous. Fever, anorexia, depression, tenesmus, colic and emaciation were also reported. While some of them revealed normal feces.

3.2. Parasitological examination of the fecal samples:

3.2.1: Morphology of the investigated *Cryptosporidium* oocyst:

The oocysts were isolated from the feces of naturally infected calves were fully sporulated by Safranin. By using light microscopic examination, the oocysts were spherical to ovoid in shape and the wall was smooth. It contains 4 sporozoites. The measurement of oocyst was varied from 3.7-5.4 x 4.5-5.6 μm (mean 4.6 x 5, 4 μm).

3.2.2. The relation between the incidence of *Cryptosporidium* infection and the seasonal variation:

Friesian calves: Concerning monthly variations of *Cryptosporidium* infection, it was found that the highest rate infection was recorded in August (60.00%), March (54.56%). and April (50.00%) The lowest rate of infection varied from 13.19% to 14.29% in February and January, respectively (Table 1). Hence, the high rate of infection was recorded in summer 46.39% followed by spring 45.65%, autumn 24.81% and winter 16.33% (Table 2). The statistical analysis of the data of Table 1 showed that the monthly incidence of cryptosporidiosis among Friesian calves in August, April and March was significant ($p < 0.05$). On the other hand, Table 2 showed that the incidence of cryptosporidiosis among calves during summer seasons was highly significant at ($p < 0.001$). Buffalo calves: Examination of buffalo calves fecal samples showed that the highest rate of infection was recorded in August (57.14%), May (40.00%) and June (31.58%). While, in December, the incidence was 10.00% (Table 3). The statistical analysis of these data showed that the monthly incidence of cryptosporidiosis among buffalo calves in August, May and June was highly significant ($P < 0.001$) (Table 4).

3.2.3. The relation between the incidence of *Cryptosporidium* infection and the age of examined animals:

Friesian calves: As shown in (Table 5) 75 and 65 out of 123, examined Friesian calves of up

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to 10 days, 11-19 days and 20-29 days only 55 (44.72), 33 (44.00%) and 28 (43.08%) were infected with *Cryptosporidium* oocysts, respectively. While it was found that out from 108 Friesian calves aged 1-3 months only 12 (11.11%) infected with *Cryptosporidium*. Buffalo calves: The present study showed that out of 97, 84 and 76 examined buffalo calves of up to 10 days, 11-19 days and 20-29 days only 28 (28.87%), 21 (25.00%) and 17 (22.37%) were infected with *Cryptosporidium* oocysts, respectively. While it was found that from 88 buffalo calves aged 1-3 months only 5 (5.68%) were infected with *cryptosporidium* (Table 5). Hence, it was concluded that animals of less than one month are more susceptible to *cryptosporidium* infection than older ones. The statistical analysis of these data showed that there was an effect of age on the incidence of cryptosporidiosis in which the incidence percentage among Friesian calves of less than month and those of 1-3 month respectively was significantly higher than that of buffalo calves of the same age ($p < 0.001$).

3.2.4. *The relation between cryptosporidium infection in diarrheic and non diarrheic calves:*

Friesian calves: The present study showed that out of 190 and 47 diarrheic calves of less than one month and 1-3 months only 107 (56.32%) and 11 (23.40%) were infected with *cryptosporidium* oocysts, respectively. While examination of 71 non diarrheic fecal material from calves of less than one month revealed that 7 (9.86%) had *cryptosporidium*. In addition, *cryptosporidium* oocysts were detected in 3 (4.76%) out of 63 non diarrheic fecal materials from calves aged 1-3 months (Table 6). Buffalo calves: Out of 182 and 44 diarrheic buffalo calves of less than one month and 1-3 months only 62 (34.07%) and 4 (9.09%) were infected with *cryptosporidium*, respectively. Also it was found that out of 78 and 42 non diarrheic

buffalo calves of less than one month and 1-3 months only 4 (5.13%) and 1 (2.38%) were infected with *cryptosporidium*, respectively (Table 6).

3.3. *The statistical analysis:*

Data showed that the incidence percentage of *cryptosporidium* oocyst shedding among diarrheic calves was significantly higher ($p < 0.001$) than that of non-diarrheic calves. On the other hand, the incidence percentage of *cryptosporidium* oocyst shedding among diarrheic and non-diarrheic Friesian calves respectively was significantly higher ($P < 0.001$) than that of diarrheic and non-diarrheic buffalo calves, respectively.

4. DISCUSSION

Cryptosporidiosis is an infectious disease of the intestinal mucosa caused by a protozoan of the enteric coccidian group. The disease was reported in a variety of mammalian species including human as well as avian species Fayer and Unger (1986). The disease caused considerable economic losses due to high morbidity among affected animals and sometimes considerable growth retardation, emaciation and poor general condition of animals Fischer (1984). The difference in the incidence of the disease might be attributed to variation in the environmental conditions (relative humidity temperature and the immune status of the investigated animals) or the variation of susceptibility of the animal breed and the age of examined animals. Regarding to the monthly variation of cryptosporidiosis, the present study clarified that the maximum rate of *cryptosporidium* infection recorded during the period from June 2012 to August 2012 was 35.5% and 60 % respectively. These results agreed with that obtained by Abdel-Salam *et al.*, (1993), Aboul-Khir, (1996) and Sharaf, (2002), in Behera, who found the highest incidence in August. While it was in contrast to that of Sanford and Josephson (1982) who stated

Table 1: The incidence of cryptosporidium infection among Friesian calves.

Months	No. of examined calves	No. of positive cases	Ratio%	Months	No. of examined calves	No. of positive cases	Ratio%
Dec 2011	13	3	23.08	Jun 2012	31	11	35.48
Jan 2012	7	1	14.21	Jul 2012	26	10	38.46
Feb 2012	29	4	13.79	Aug 2012	40	24	60.00
Mar 2012	55	30	54.55	Sep 2012	51	8	15.69
Apr 2012	14	7	50.00	Oct 2012	56	17	30.36
May 2012	23	5	21.74	Nov 2012	26	8	30.17

Table 2: The seasonal incidence of cryptosporidium infection among Friesian calves.

Season	No. of cow calves samples	No. of positive cases	Ratio%
Winter	49	8	16.32
Spring	92	42	45.65
Summer	97	45	46.39
Autumn	133	33	24.81
Total	371	128	34.50

Table 3: The incidence of cryptosporidium infection among buffalo calves collected during the period from December 2011 to November 2012.

Months	No. of examined calves	No. of positive cases	Ratio%	Months	No. of examined calves	No. of positive cases	Ratio%
Dec 2011	30	3	10.006.7	Jun 2012	38	12	31.58
Jan 2012	15	3	20.00	Jul 2012	30	0	0.00
Feb 2012	24	0	0.00	Aug 2012	49	28	57.14
Mar 2012	20	0	0.00	Sep 2012	20	0	0.00
Apr 2012	10	0	0.00	Oct 2012	40	8	20.00
May 2012	25	10	40.00	Nov 2012	45	7	15.56

Table 4: The seasonal incidence of cryptosporidium infection among buffalo calves

Season	No. of cow calves samples	No. of positive cases	Ratio%
Winter	69	6	8.707.2
Spring	55	10	18.18
Summer	117	40	34.19
Autumn	105	15	14.29
Total	346	71	20.52

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Table 5: The incidence of cryptosporidium infection among calves in relation to age.

Age	Frisian calves			Buffalo calves		
	Examined calves	No. of positive cases	Ratio%	Examined calves	No. of positive cases	Ratio%
1-10 day	123	55	44.72	97	28	28.7
11-19 day	75	33	44.00	84	21	25.00
20-29 day	65	28	43.08	76	17	22.37
1-2 m	60	8	13.33	50	3	6.00
2-3 m	48	4	8.33	38	2	5.26
1-3 m	108	12	11.11	88	5	5.68

Table 6: The incidence of cryptosporidium infection among calves in relation to fecal consistency

Calves	Age	Diarrheic			Non diarrheic		
		Examined calves	+ve	%	Examined calves	+ve	%
Friesian	1-30 day	190	107	56.32	71	7	9.86
	1-2 m	30	8	26.67	39	2	5.13
	2-3 m	17	3	17.65	24	1	4.17
	1-3 m	47	11	23.40	63	3	4.76
Buffalo	1-30 day	182	62	34.07	78	4	5.13
	1-2 m	29	3	10.34	30	1	3.33
	2-3 m	15	1	6.67	12	0	0.00
	1-3 m	44	4	9.09	42	1	2.38

that 69% of cryptosporidium infected calves were submitted between December and February. Also, it was recorded that the highest incidence of infection was during short dry season (December-January) Bogaerts, *et al.*, (1987). Concerning to the seasonal variation of cryptosporidium infection among calves, the data revealed that the highest rate of infection was in summer (47%) followed by spring (42%), autumn (18.2%) and winter (18%), these results were in agreement to that recorded by Abou-Eisha *et al.*, (2000), Scott *et al.*, (1994), Salem, (1989) , Sharaf (2002) in Behera. While Sanford and Josephson (1982) contrasted with the prior work who found that, the incidence was higher in autumn than summer, spring and winter. Also, Shokier (1999) and Al-Khodery, (1996) mentioned that the highest rate of infection was in winter followed by spring, autumn and summer. However, Ongerth and Stibbs (1989) reported that there was no distinct seasonal variation. The incidence of cryptosporidiosis among

calves during summer season was highly significant. This again could be attributed to high temperature and high humidity. As regard to the age susceptibility in calves. The obtained results showed that the infection rate in cattle calves of up to 10 days, 11-19 days, 20-29 days and 1-3 months were 43%, 42.7%, 41.5% and 10.2%, respectively. While in buffalo calves of up to 10 days, 11-19 days, 20-29 days and 1-3 months were 29%, 25%, 22.5% and 5.7%, respectively. Such data indicated that, the rate of cryptosporidium infection was higher in the young age and gradually decreased in older groups. This finding agreed with that of Ongerth and Stibbs (1989) Who found that 42-51% of 7-21 days old calves had cryptosporidium (Abdel-Salam *et al.*, 1993 and Sharaf, 2002) in Behera who found that, 15.4% and 0.6% of cryptosporidial positive cases were among one month old calves and 2 years old cows. However, Villacorta, *et al.*, (1991) proved that the infection was also recorded in adult cattle. While Androsen, (1982) failed to

detect cryptosporidium oocysts in 1500 fecal samples collected from adult cows. On the other hand, the above results recorded in buffalo calves agreed with that of Iskander *et al.*, (1987) who reported that the infection rate was very high (21%) in ages ranging between 2-12 days among diarrheic buffalo calves (Salem, 1989) detected 13.6% and 4.2% cryptosporidial positive. Buffalo calves among one week and 12 weeks old, respectively. Abou-Eisha *et al.*, (2000) Who mentioned that 13.3% of buffalo calves of less than one month had cryptosporidium and Aboul-Khir (1996) found that 7.22% of buffalo calves of up to 2 months had cryptosporidium. While Shokier (1999) found that the incidence of infection was 46.6% in ages of less than one month and showed that the incidence of cryptosporidiosis among buffalo calves of different age lower than that of Friesian calves of the same age. This again could be attributed to species and genetic susceptibility. Divers and Peek (2008) and Foster and Smith (2009) reported that, the infection rate among young calves aged older aged. The present study showed that cryptosporidium infection could be considered as a causative agent of diarrhea in calves as 47.25% of diarrheic calves had cryptosporidium. The same observation was obtained by Abou-Eisha, *et al.*, (2000); El-Masry, (1996) and Naciri, *et al.*, (1999) while cryptosporidium infection was 6% in non-diarrheic calves. The achieved results almost coincide with those obtained by Abou-Eisha, *et al.*, (2000); Al-Khodery (1996) and Sharaf, (2002) in Behera who recorded

cryptosporidium in apparently healthy calves with infection rates of 6.35%, 4.5%, 7.84% and 7.7% respectively. While El-Masry (1996) detected cryptosporidium infection in 37.5% and 18.18% in diarrheic and non-diarrheic calves, respectively. On the contrast Sobih and Sabah (1987); O'Hara, and Chen (2011) stated that the infection rate of cryptosporidium infection among diarrheic buffalo calves aged one month old were 33%. While it was 3% in non diarrheic buffalo calves aged one month old and Coklin, *et al.*, (2009) reported that an infected calves sheds several million oocysts in the diarrheic feces (up to 107). The concentration and risk of infection in an affected farm is very high 13 (infective pressure) and the problem can easily turn into a herd problem (O'Donoghue, 1995 and Hamnes, *et al.*, 2006) found that calves infested with cryptosporidium parvum had a significantly higher rate of diarrhea than non-infested calves of that none of the examined clinically normal calves were harboring cryptosporidium oocysts. From the above mentioned data it could be concluded that the incidence of cryptosporidiosis among diarrheic calves was significantly ($p < 0.001$) higher than that of non diarrheic calves. On the other hand, the same table and figure showed that diarrheic and non diarrheic buffalo calves had an incidence percentage of 28.3% and 4.16% respectively as compared to diarrheic and non diarrheic Friesian calves which had an incidence percentage of 47.25% and 6%. This difference was significant. This again could be attributed to species and genetic susceptibility.

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معدل الإصابة بالكريبتوسبورديوم بين العجول الفرزيان والجاموس في محافظة المنوفية

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³قسم الطفيليات، كلية الطب البيطرى ، جامعة بنها

الملخص العربي

أجريت هذه الدراسة لدراسة معدل الإصابة بالكريبتوسبورديوم بين عجول الفرزيان والجاموس في محافظة المنوفية. وجد أنه تم تسجيل ارتفاع معدل الإصابة بالكريبتوسبورديوم في أغسطس (60%)، إبريل (54.54%)، ومارس (50%). تم تسجيل انخفاض معدل الإصابة في سبتمبر 2012 إلى فبراير 2012 (1569% - 1379%). أظهرت التغيرات الموسمية التي تم تسجيلها أعلى معدل للعدوى في فصل الصيف (46.39%)، يليه الربيع (45.65%)، خريف (24.81%)، والشتاء (16.35%). وأظهرت الدراسة أن معدل الإصابة بالكريبتوسبورديوم بين العجول 44.72%، 44%، 43.08%، و11.11% والعجول الفرزيان من الذين تصل أعمارهم إلى 10 أيام، 11-19، 20-29 يوماً، 1-3 أشهر على التوالي. أيضاً، كان 28.87%، 25%، 22.37%، 5.68% بين عجول الجاموس الذين تصل أعمارهم إلى 10 يوماً، أيام 11-19، 20-29 يوماً، 1-3 أشهر على التوالي. كشفت الدراسة أن معدل الإصابة بين العجول المصابة بالإسهال أقل من شهر واحد كان 56.32%، 23.40% الذين تتراوح أعمارهم بين 1-3 أشهر، بينما كانت العدوى بين العجول الاصحاء 9.86% - 4.76% والذين تتراوح أعمارهم بين أقل من شهر واحد و 1-3 أشهر على التوالي. وبالإضافة إلى ذلك، فإن حالات في عجول الجاموس 34.07% - 9.09% وعجول الجاموس في الإسهال الذين تقل أعمارهم عن شهر واحد و 1-3 أشهر على التوالي. بينما في عجول الجاموس غير الإسهال وحالات 5.13% و 2.38% في عجول الجاموس الذين تتراوح أعمارهم بين أقل من واحد و 1-3 أشهر على التوالي

(مجلة بنها للعلوم الطبية البيطرية: عدد 26 (1):195-204, مارس 2014)