

FIELD STUDY ON THE MOST IMPORTANT PROTOZOAL CAUSES OF DIARRHOEA IN CALVES AND THEIR TREATMENT

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SUMMARY

The present study was conducted on 218 Friesian calves, out of them 131 calves were suffering from diarrhea and 87 calves were apparently healthy. Faecal samples were collected from calves in different localities of Egypt (Middle Delta, West Delta and Upper Egypt). The parasitological examination (Macroscopic and Microscopic of the collected Faecal samples revealed that, the prevalence of *Cryptosporidium* and *Giardia* was 50.4% and 20.7%, of either diarrheic or apparently healthy calves respectively. The differences in the prevalence of *Cryptosporidium* and *Giardia* influenced by seasonal variations, the age of examined calves and geographical regions. The neonatal calves aged from birth till 2 weeks and 2 - 4 weeks old were highly susceptible to the parasitic infestation at the rate of 59.5% and 67.9%, respectively. The percent of recovery from cryptosporidiosis and giardiasis in diarrheic calves was 80 % when treated with Nitazoxanide only, while it reached to 100 % when the drug of treatment was accompanied with therapeutic supportive solutions (saline and ringers).

Keywords:

Prevalence, *Cryptosporidium*, *Giardia* diarrhea, Egypt, season, age and geographical regions

INTRODUCTION

Neonatal diarrhoea is a major cause of economic losses in the cattle industry and a predisposing cause of calf mortality in most countries. The economic losses associated with this disease were not only due to the resulting mortality, but also to the retarded growth of the animals, the cost of drugs, veterinary assistance and the increased labor involved (**Mallinath *et al*, 2009**). Calf scour is not a single disease entity; it is a clinical syndrome associated with several diseases characterized by diarrhoea. Cryptosporidiosis and Giardiasis are diarrheal diseases caused by microscopic parasites that can live in the intestine of animals and are passed in the stool of an infected animal. (**Fayer, *et al.*, 2000**).

Cryptosporidiosis, especially in calves, has been associated with a wide range of clinical signs from no apparent ill effects to severe morbidity, resulting in poor performance and production losses and in some instances mortality. The severity of cryptosporidiosis symptoms is depending on the site of infection, nutritional and immune status of the host. Shedding of *Cryptosporidium* oocysts in the stool begins when the symptoms begin and can last for weeks after the symptoms (e.g., diarrhoea) stop. (Mallinath *et al*, 2009). Giardiasis is a zoonotic parasitic disease and considered as one of the most common pathogenic parasitic infestation of a wide variety of domestic and wild animal species. Cryptosporidiosis and giardiasis are transmitted via the faecal-oral route with the ingestion of contaminated water and food with oocysts (Klein, *et al* 2008). The present work was designed as a field study for determination of the most important protozoal causes of diarrhoea (*Cryptosporidium* and *Giardia*) in calves with their treatment trials. Besides, the relation of their prevalence's in relation to seasonal and localities variation as well as the age susceptibility.

MATERIALS AND METHODS

Calves:

This study was conducted on 218 Friesian calves, out of them 131 calves suffered from persistent diarrhea, pasty-watery faces, poor appetite, reduced suckle reflex and dehydration; and 87 calves were apparently healthy calves (no signs of diarrhea).

The examined calves were found in different localities of Egypt (Middle Delta, West Delta and Upper Egypt). These calves were fed on the whole milk and were exposed to full clinical and parasitological examinations.

The examined calves were classified into 5 groups as follows: based on age of calves,

Group 1: this group consisted of 52 calves aged from 0 - 2 weeks.

Group 2: this group consisted of 43 calves aged from 2 - 4 weeks.

Group 3: this group consisted of 42 calves aged from 1 - 3 months.

Group 4: this group consisted of 45 calves aged from 3 - 6 months.

Group 5: this group consisted of 36 calves aged more than 6 months.

Faecal samples:

Approximately 20 mL of fecal material was collected in sterile containers directly from the rectum of diarrheic calves by direct digital stimulation using disposable latex gloves. Samples were then kept in ice box till and transferred to the laboratory. All samples were then centrifuged and the resulted supernatant was collected in sterile containers, and kept at -20°C.

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Parasitological examination of the faecal samples:

a- Macroscopic examination:

It was applied to detect the abnormalities in consistency, colour of the faeces, mucous, blood, frank adult worms and any other abnormalities.

b- Microscopic examination:

The faecal samples were examined by ordinary direct smear method and sedimentation by using saline. These methods were performed according to Belding, (1952) to examine the provided samples for presence of vegetative and cysts form of protozoal parasites as well as any other GIT ova.

C-Staining procedures:

Smears were prepared from faecal samples. After air drying, smears were stained by Ziehl-Neelsen staining technique according to **Henricksen and Pohlenz (1981)**.

Treatment regime:

2 groups of severe diarrheic calves, each group consisted of 15 calves, the first group received the Nitazoxanide suspension is 500 mg / calves in 2 doses for 3 successive days. The second group was given combinations of the drug with rehydration solutions (electrolyte replacement) as saline and ringers.

RESULTS

Table (1): Distribution of protozoal infestation in relation to seasonal variations in infested calves in Egypt.

Season	Examined samples	Diarrheic		App. healthy		Total
Summer	72	40	22 (55%)	32	8 (25 %)	30 (41.7 %)
Spring	60	30	19 (63.3%)	30	6 (20 %)	25 (41.7 %)
Winter	55	37	16 (43.2%)	18	2 (11.1%)	17 (32.7 %)
Autumn	31	24	9 (37.5%)	7	2 (28.6%)	11 (35.5 %)
Total	218	131	66 (50.4%)	87	18 (20.7%)	84 (38.5 %)

Table (2): The relation between the incidences of protozoal infestation and age of in infested calves.

Age	Total No. of examined calves	Diarrheic calves		App. healthy calves		Total
		No. of examined	positive cases	No. of examined	positive cases	
0 - 2 weeks	52	37	22 (59.5%)	15	2 (13.3%)	24 (46.2%)
2 - 4 weeks	43	28	19 (67.9%)	15	4 (26.7%)	23 (53.5%)
1 - 3 months	42	23	7 (30.4%)	19	6 (31.6%)	13 (31 %)
3 - 6 months	45	23	11 (47.8%)	22	3 (13.6%)	14 (31.1%)
months↑6	36	20	7 (35 %)	16	3 (18.8%)	10 (27.8%)
Total	218	131	66 (50.4%)	87	18 (20.7%)	84 (38.5%)

Table (3): Distribution of protozoal infestation in relation to geographical regions in infested calves in Egypt.

region	No. of infested samples		Middle Delta		West Delta		Uper Egypt	
	dia	App	dia	App.	dia	App.	dia	App.
0 - 2 weeks	22	2	13 (59.1 %)	1 (50 %)	5 (22.7 %)	1 (50 %)	4 (18.2 %)	0
2 - 4 weeks	19	4	12 (63.2 %)	2 (50 %)	3 (15.8 %)	1 (25 %)	4 (21.1 %)	1 (25 %)
1 - 3 months	7	6	3 (42.9 %)	2 (33.3 %)	3 (42.9 %)	3 (50 %)	1 (14.3 %)	1 (16.7%)
3- 6 months	11	3	5 (45.5 %)	1 (33.3 %)	5 (45.5 %)	2 (66.7 %)	1 (20 %)	0
↑ 6 month	7	3	3 (42.9 %)	0	2 (28.6 %)	2 (66.7 %)	2 (28.6 %)	1 (33.3 %)
Total	66	18	36 (54.5 %)	6 (33.3 %)	18 (27.3 %)	9 (50 %)	12 (18.2 %)	3 (16.7 %)

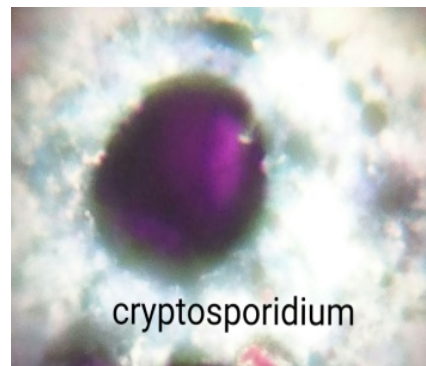
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Table (4): Differentiation of single and mixed infestations of Cryptosporidium and Giardia in infested calves.

	Parasite	Diarrheic	App. Healthy	TOTAL
Single infestation	Crypto	38 (57.6 %)	8 (44.4 %)	46 (54.8 %)
	Giardia	16 (24.2 %)	6 (33.4 %)	22 (26.1 %)
Mixed	C & G	12 (18.2 %)	4 (22.2 %)	16 (19.1 %)
TOTAL		66	18	84

Table (5): Results of treatment of infested calves with Cryptosporidium and Giardia.

	Diarrheic calves	Cured calves	% of cured calves
Nitazoxanide	15	12	(80 %)
Nitazoxanide and supportive Treatment	15	15	100 %



DISCUSSION

Cryptosporidium and Giardia infections have been reported in cattle worldwide. Before the development and application of molecular methods to aid in species determination, numerous publications simply documented the presence of Cryptosporidium and Giardia oocysts in calves' faeces. Subsequently, many other publications have identified Cryptosporidium species in cattle and calves but with limited information on prevalence and herd management. Cryptosporidiosis, especially in calves, has been associated with a wide range of clinical signs from no apparent ill effects to severe morbidity, resulting in poor performance and production losses and in some instances mortality (Radostits *et al.*, 2007). Moreover, the Cryptosporidium and Giardia are worldwide causes of enteric infections in both humans and animals, and, due to the zoonotic character of some of their species, they are among the most relevant parasitic enteric agents in human and veterinary medicine (Esch and Petersen, 2013) and (Gulliksen *et al.*, 2009). Cryptosporidiosis is an infectious disease of the intestinal mucosa caused by a protozoan of the enteric coccidian group, (Fayer and Unger, 1986). The diseases cause considerable economic losses due to high morbidity among affected animals and sometimes considerable growth retardation, emaciation and poor general condition of animals (Fischer, 1984). In the present study, 84 out of 218 (38.5%) of examined faecal samples collected from calves, either diarrhoeic or apparently healthy calves, were infested with Cryptosporidium and Giardia. These results were in parallel to that reported by El-Dessouky and El-Masry, (2005) who recorded that, the percent of isolated cryptosporidium in dairy young calves (30.2%), and higher than El-Sherif, *et al.*, (2000); Amer, *et al.*, (2010) and El-Khodery and Osman, (2008) who recorded that, the percent of isolated cryptosporidium in buffalo and dairy young calves were (15.6%), (21.7%) and (22.4%) respectively but lower than (95%) estimated by Ramirez *et al.*, (2004) in US dairy farms and (52%) which had been reported by Silverlås, *et al.*, (2009) in European young calves. From the tabulated results (Tables 1, 2), it is evident that Cryptosporidium and Giardia were isolated from both diarrhoeic and apparently healthy calves with no signs of diarrhea in ratio of 50.4% and 20.7%, respectively. It was found that, the higher prevalence of cryptosporidiosis was in diarrhoeic calves compared to non-diarrhoeic animals, these results agreed with many authors as Singh, *et al.*, (2006) and Lin *et al.*, (2012). Shedding of Cryptosporidium and Giardia in the stool begins when the symptoms begin and can last for weeks after the symptoms (e.g., diarrhoea) stop. Cryptosporidium may be found in soil, food, water, or

surfaces that have been contaminated with the faeces from infected humans or animals. These factors can explain the prevalence of *Cryptosporidium* and *Giardia* in non-diarrheic calves. Moreover, *Giardia* oocysts can stay infectious for up to three months in adverse condition e.g. cold water (**Youssef, et al., 2008**) and not all *Giardia* infections are symptomatic. Many animals and people can unknowingly serve as carriers of the parasites, (**Abd-El-Wahed, 1999**). Several factors may be responsible for the differences in the prevalence of *Cryptosporidium* and *Giardia* in the present study as seasonal variations (Table 1), the age of examined calves (Table 2) and geographical regions (Table 3). Regarding seasonal variations, the results in (Table 1) showed that, the prevalence of *Cryptosporidium* and *Giardia* infestations in calves were higher in Summer and Spring as they were (55%) and (25%) for diarrhoeic and apparently healthy calves, respectively in Summer season and (63.3%) and (20%) for diarrhoeic and apparently healthy calves, respectively in Spring while in Winter they were (43.2%) and (11.1%) and in Autumn were (37.5%) and (28.6%) for diarrhoeic and apparently healthy calves, respectively. The overall prevalence of infested calves were (41.7%), (41.7%), (32.7%) and (35.5%) in summer, spring, winter and autumn, respectively. These results agreed with that obtained by **Abdel-Salam et al., (1993)**, **Aboul-Khir, (1996)** and **Sharaf, (2002)**, who found the highest incidence in August; while it was in contrast to that reported by, **Razzaque et al., (2006)**. The higher incidence of *Cryptosporidium* infestations in calves during summer and spring may be owing firstly to resistant *Cryptosporidium* oocysts that pass from the host into the environment and are infective and need no time to mature. Therefore they may not be affected much by harsh environmental conditions as they are directly transmitted to calves. The widespread of *Cryptosporidium* in calves although the weather is extremely hot and inhospitable to any pathogenic agents, Secondly, poor husbandry and management and housing systems are factors affecting the spread of the parasites. A relationship existed between the age of the calves and the prevalence of infestations with *Cryptosporidium* and *Giardia* species was observed and illustrated in (Table 2). The results revealed that neonatal diarrheic calves of 2-4 weeks old and those aged from birth till 2 weeks were highly susceptible to contract the parasitic infestation with *Cryptosporidium* and *Giardia* as their prevalence were (67.9%) and (59.5%), respectively. In parallel, their prevalence was higher in both diarrheic and apparently healthy calves of the same age groups, (53.5%) and (46.2%), respectively. These results were in harmony with conclusions reported by **Olson, et al., (2004)**, **Fayer and Xiao, (2008)** and

Ozdal, *et al.*, (2009), as they concluded that *Cryptosporidium* spp. was one of the most frequent pathogens responsible for outbreaks of severe diarrhoea, mainly in calves up to one month of age. The difference in the incidence of the disease might be highly dependent on the immune status of the investigated calves or the variation in susceptibility of the calves breed and the age of examined animals. In immuno-competent individuals *Cryptosporidium* infections most commonly result in acute self-limiting gastroenteritis, cryptosporidiosis in immuno-compromised individuals can develop into a chronic and life-threatening diarrheal disease, Kvac *et al.*, (2006) and Duranti, *et al.*, (2009). Additionally, geographical (locality) variations are important factors affecting the prevalence of *Cryptosporidium* and *Giardia* and their severity of infection as shown in (Table 3), where it was (54.5%), (27.3%) and (18.2%) in diarrhoeic calves in Middle Delta, West Delta and Upper Egypt, respectively, while in apparently healthy calves the prevalence rates were (33.3%), (50%) and (16.7%), respectively. These variations were attributed to the variation in the environmental conditions (relative humidity and temperature), since the hot dry weather as in Upper Egypt region is considered as a physical means of disinfection leading to a decrease in the incidence of exposure of calves to new infestations with infective oocysts. On the other hand, rains accompanied with low temperature help in making muddy soil in calves rearing areas, which in turn causing favorable condition for viability and infectivity of the protozoa (Osman and Sadiq, 2008 and El-Khodery and Osman, 2008). Bad hygienic measures, poor husbandry and inadequate and/or poor quality of drinking water, some of these factors may act individually or collectively to increase the risk factor associated with transmission and prevalence of protozoal infestation between calves. (Table 4) revealed that, the majority of those protozoal infestations in the examined calves, either diarrhoeic or apparently healthy, were recorded as single infestations. Out of 84 infested calves, 46 calves were infested with *Cryptosporidium* only (54.8%), and 22 ones were infested with *Giardia* only, (26.1%). While 16 examined calves (19.1%) have contracted both *Cryptosporidium* and *Giardia*. This finding could be attributed to different factors as diversity of diarrheic calves' natural immunity and their susceptibilities to attract mixed infection, as well as severity of infection, hygienic measures, breed of the calves and season of examination (Malik, *et al.*, 2012). Depending on the approval of using Nitazoxanide in treatment of diarrhoeic animals caused by protozoal infestations especially cryptosporidiosis, the drugs were used in treatment of severe cases of cryptosporidiosis (Schlagenhauf *et al.*, 2015) and Abubakar *et al.*, 2007). The results

illustrated in (Table 5) declared the effectiveness of Nitazoxanide in treatment of cryptosporidiosis and giardiasis in diarrhoeic calves where (80 %) of diarrhoeic calves recovered from the disease when treated with Nitazoxanide only. The percent of recovery from the disease was enforced, reached to (100 %) when the drug treatment accompanied with therapeutic supportive solutions as saline, ringers solutions.

CONCLUSION

In conclusion, *Cryptosporidium* and *Giardia* are parasites which have the ability to induce severe diarrhoea in neonatal and young calves. Therefore, their importance as a cause of diarrhoea in calves and their adverse effects on animal health and wealth should be put in our consideration. Moreover, their remedy is easy and possible with using suitable anti-protozoal drugs as Nitazoxanide apart from the exaggerated usage of antibiotics.

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