Global Veterinaria 13 (5): 662-667, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.05.8695

Prevalence of *Cryptosporidium* Infection in Buffalo Calves with Special Reference to Urea and Creatinine Levels

[1]G.H. Morsy, [2]Kadria N. Abdel Megeed, [3]A.M. Hammam,
[1]M.M.E. Seliem, [2]Fathia A.M. Khalil and [2]Dina Aboelsoued

[1]Department of Zoology, Faculty of Science, Benha University, Benha, Egypt
[2]Department of Parasitology and Animal Diseases, National Research Centre, Dokki, Giza, Egypt
[3]Department of Animal Reproduction and A.I., National Research Centre, Dokki, Giza, Egypt

Abstract: The present study was conducted on 571 buffalo calves from different Egyptian governorates (Cairo, Giza, Beni Suef and Qualiobya) around the year. Four age groups were involved; less than one month, from 1-2 months, from 2-6 months and more than 6 months of age. Modified Ziel-Neelsen staining technique (MZN) was used for the detection of *Cryptosporidium* oocysts in formed and diarrheic fecal samples. Blood samples were collected for biochemical analysis. Prevalence of *Cryptosporidium* infection among buffalo calves was 52%. The highest rate of infection was in animals aged less than 1 month (62%) The lowest rate of infection was reported in buffalo calves aged more than 6 months (46%). Regarding seasonal prevalence, *Cryptosporidium* infection was higher in spring (58%) followed by winter (55%) then, autumn (50%) while, the lowest rate was in summer (43%). Dealing with biochemical changes, the infected animals showed significant increase in serum urea and creatinine than healthy ones. In conclusion, Cryptosporidiosis had a relationship with age and seasonal variations. Also, it had an adverse effect on biochemical parameters of infected buffalo-calves.

Key words: Buffalo Calves · *Cryptosporidium* · Prevalence · Urea · Creatinine

INTRODUCTION

Cryptosporidium is a protozoan parasite of the Apicomplexan genus and is recognized to be infecting a wide range of host species including mammals, birds, reptiles and fish [1]. Transmission was mainly by ingestion of sporulated oocysts through contaminated food and water [2]. 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 [3]. Cryptosporidiosis is the clinical disease, usually presenting as a gastro-enteritis-like syndrome ranging from mild to severe symptoms depending on the site of infection, nutritional and immune status of the host [4]. *Cryptosporidium spp.* was one of the most frequent pathogens responsible for outbreaks of severe diarrhea, mainly in calves up to one month of age [5&6]. A relationship existed between the age of the animal and the species of *Cryptosporidium* [7]. Most *Cryptosporidium* infections in pre-weaned calves were due to *C. parvum* and those in post-weaned calves were due to *C. bovis* and *C. ryanae. C. andersoni* was most often found in cattle over 1 year of age [8]. Fecal specimens of calves revealed a percent of 19.2% *Cryptosporidium*-positive calves. The highest prevalence of infection was at two weeks of age when 29 of the 30 calves were excreting oocysts. In a dairy farm in Maryland, USA, prevalence was higher in pre-weaned calves (1-8 weeks of age) (45.8%) than in post-weaned calves (3-12 months of age) (18.5%) and heifers (12-24 months of age) (2.2%) [9].

In Egypt, *Cryptosporidium* was responsible for considerable part of diarrheal illness among American military personnel participated in a military exercise in the northwestern Egyptian desert at the fall of 2001 [10].Cryptosporidiosis was reported in Egypt in farm animals as well as in domestic animals [11 & 12].

Corresponding Author: Dina Aboelsoued, Department of Parasitology and Animal Diseases, National Research Centre, Dokki, Giza, Egypt.

Also, human cryptosporidiosis was reported in different ages [13]. Regarding locations, *Cryptosporidium* infection was detected in Qualubia Governorate with a prevalence (30% in lambs) [14], El Dakahlia (19.65% in buffaloes) [15], The northwestern Egyptian desert (7% in humans) [10], Delta (17% in children) [16], Fayoum (15% in children) [17], Assuit (21% in calves) [18], Middle Egypt (14.19% in buffalo calves) [12] and Ismailia (20.9% in sheep, 22.5% in buffaloes, 23.7 in cows and 25.9% in goats) [19].

The biochemical analysis of buffalo calves sera infected with *C. parvum* revealed a significant increase in serum urea and creatinine levels [15]. On the other hand, others detected a significant increase in serum urea levels with insignificant increase in serum creatinine [18].

Little attempts had been introduced concerning the course of Cryptosporidiosis in buffalo calves and its effects on animal biochemical parameters. The present work aimed to study the prevalence of *Cryptosporidium Spp.* in some localities of Egypt around the year and the relationship between the infection and animal age. Also, it aimed to study the effect of the infection on urea and creatinine levels.

MATERIALS AND METHODS

Animals: A total of 571 buffalo calves samples were collected around the year from different Governorates (Cairo, Giza, Beni Suef, Qualiobya). Four age groups were involved; less than one month, from 1-2 months, from 2-6 months and more than 6 months of age.

Sample Collection: Fecal samples were collected from animal's rectum in a separate clean labeled container. Formed and diarrheic samples were tested.

Blood samples were collected from each examined animal in a dry sterile tube, centrifuged at 3000 rpm and separated in small tubes then labeled, dated and frozen at -20°C for biochemical analysis.

Detection of Oocysts: Fine fecal smears fixed with methanol spirit and stained with Modified Ziehl-Neelsen Stain (MZN) [20]. The oocysts were measured with help of stage micrometer conjugated with the light microscope at the eyepiece 10x and the objective 100x. All measurements are in micrometers (µm) for about 20-50 oocysts [21].

Determination of Urea and Creatinine: Serum urea and creatinine levels were estimated using test kits supplied by Bio diagnostic Co., Giza, Egypt [22 & 23].

Statistical Analysis: Data of serum biochemistry were analyzed for the means and standard deviations. Significance of the results was evaluated using Independent sample t-test, Statistical Package for Social Science (SPSS) computer programs.

RESULTS

Morphology of the Detected *Cryptosporidium Parvum* Oocysts: The detected *Cryptosporidium* oocysts in the examined calve feces stained with Ziehl-Neelsen technique were morphologically similar to *Cryptosporidium parvum* oocysts which characterized by spherical to ovoid shape with smooth wall and appeared as acid fast (red-pink) on green back. The measurements of 50 oocysts were varied from 4.4- 5.8 x 4.3 - 4.9 µm of mean (5.1 x 4.6) and the shape index was 1.0-1.2 of mean (1.1) (Fig. 1).

Seasonal Prevalence of *Cryptosporidium* Infection among Buffalo-calves in Different Age Groups and Fecal Consistency: Regarding seasonal prevalence, *Cryptosporidium* infection was higher in spring (58%) followed by winter (55%) then, came autumn (50%) while, the lowest rate was in summer (43%). Considering age of animals, the highest rate of infection was in animals aged from 1-30 days (62%) where, (66%) was for diarrheic samples and (48%) for formed ones followed by 52% prevalence in animals aged from 1-2 months where, 47% was for diarrheic samples and 61% for formed ones. Then, the percentage of *Cryptosporidium* infection in buffalo calves aged from 2-6 months was 49%. It was 60% for diarrheic animals and 40% for formed ones. The lowest rate of infection was reported in buffalo calves aged more than 6 months (46%) in which 53% was for diarrheic buffalo calves samples and 41% for formed ones (Tables 1 & 2 & 3).

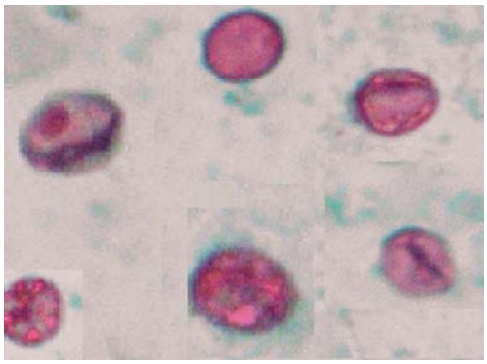

Fig 1: *Cryptosporidium* oocysts in stained fecal smears of buffalo calves (X100).

Table 1: Seasonal prevalence of *Cryptosporidium* infection among buffalo-calves in different age groups

	Age Group														
	Less than 1 month			1-2 months			2-6 months			More than 6 m					
Months	N	+ve	%	N	+ve	%	N	+ve	%	N	+ve	%	Total N	+ve	%
Winter	31	21	68%	12	5	42%	57	36	63%	47	19	40%	147	81	55%
Spring	41	26	63%	27	13	48%	28	18	64%	40	22	55%	136	79	58%
Summer	34	23	68%	28	14	50%	32	11	34%	51	15	29%	145	63	43%
Autumn	26	12	46%	34	21	62%	46	15	33%	37	24	65%	143	72	50%
Total	132	82	62%	101	53	52%	163	80	49%	175	80	46%	571	295	52%

N: number of buffalo calves examined

+ve: number of *Cryptosporidium* infected buffalo calves

Table 2: Relation between *Cryptosporidium* infection and fecal consistency by MZN technique in different age groups:

	Diarrhea			Formed		
Age Group	Number of examined animals	*Cryptosporidium* infected animals	%	Number of examined animals	*Cryptosporidium* infected animals	%
Less than 1 month	101	67	66%	31	15	48%
1-2 months	60	28	47%	41	25	61%
2-6 months	73	44	60%	90	36	40%
More than 6 months	72	38	53%	103	42	41%
Total	306	177	58%	265	118	45%

Table 3: Prevalence of *Cryptosporidium* infection in buffalo-calves around the year and fecal consistency:

	Fecal Consistency					
	Diarrhea			Formed		
Months	Number of examined animals	*Cryptosporidium* infected animals	%	Number of examined animals	*Cryptosporidium* infected animals	%
January	39	30	77%	5	5	100%
February	30	17	57%	24	7	29%
March	17	8	47%	32	14	44%
April	16	7	44%	25	19	76%
May	23	9	39%	21	9	43%
June	27	20	74%	24	15	63%
July	20	7	35%	20	9	45%
August	26	12	46%	23	12	52%
September	22	18	82%	34	5	15%
October	24	16	67%	23	6	26%
November	27	15	56%	17	11	65%
December	35	18	51%	17	6	35%
Total	306	177	58%	265	118	45%

Table 4: Mean values of urea and creatinine concentrations in apparently healthy and *Cryptosporidium* infected buffalo calves

Parameters	Apparently healthy buffalo calves	*Cryptosporidium* infected buffalo calves
Serum Urea Concentration (mg/dl)	21.52 ± 1.95	25.62 ± 2.6*
Serum Creatinine Concentration (mg/dl)	0.86 ± 0.03	0.92 ± 0.02*

Values represented by Means ± Standard deviation.

*$P < 0.05$ indicates significance.

Urea and Creatinine Concentration Changes in Apparently Healthy and *Cryptosporidium* Infected Buffalo Calves: Regarding biochemical changes, there was a significant increase in serum urea and creatinine in *cryptosporidium* infected animals than healthy ones (Table 4).

DISCUSSION

In the present study 297 out of 571 (52%) buffalo calves investigated were confirmed by acid fast stain to be infected with *Cryptosporidium*. These results were higher than (15.6, 21.7, 22.4 and 30.2%) detected by previous studies [24, 25, 15 & 12] in Egyptian buffalo calves and dairy calves, respectively; but lower than (95%) estimated by Ramirez *et al*. [26] in US dairy farms; whereas, these results are consistent with (52%) which had been reported by Silverlås *et al*. [27]in European young calves. Several factors may be responsible for the differences in the prevalence from the present study to those studies, such as severity of infection, hygienic measures, breed of the calves and season of examination. Some of these factors may act individually or collectively to increase the risk factor associated with transmission and prevalence of *Cryptosporidium* between calves [28 & 29].

Animal age played a great role in calf susceptibility to *cryptosporidium* infection in this study. It has been observed that the high prevalence (62%) of cryptosporidiosis in this study was detected in calves less than one month followed by animals aged 30-60 days (52%) then the animals aged from 2-6 months (49%) while, animals aged more than 6 months had the lowest prevalence of *Cryptosporidium* infection (46%). These findings were agreed with that revealed the high degree of infection (11.7-15.6%) in individuals below 1 month of age and then gradually decrease with age to reach 0% in those over 4 months old [24, 30-36]. Several studies have demonstrated that only pre-weaned calves are important sources of zoonotic cryptosporidiosis in humans [7], that calves less than 2 months of age were the major contributors of zoonotic *C. parvum* [28& 37]. Also, the highest prevalence was at 2 weeks of age (96.7%) then at 1-8 weeks (45.8%) then at 3-12 months (18.5%) and finally the lowest prevalence was in animals aged 12-24 months (2.2%) [38].

It was found that the higher prevalence of cryptosporidiosis was in diarrheic calves compared to non-diarrheic animals, these results agreed with many authors [39-41]. Regarding seasonal prevalence, *Cryptosporidium* infection was higher in spring (58%) followed by winter (55%) then autumn (50%) while, the lowest rate was in summer (43%). These results partially agreed with some authors [24, 42] and other investigators [43&44] had detected that the highest prevalence was in winter season followed by spring, autumn and summer. These differences could be attributed to the differences between localities and weather (humidity and temperature). Although some authors had observed an increase in the prevalence of this infection during certain seasons, in relation to high rainfall or the number of births, seasonal effects could only be correctly evaluated if the studies were repeated over several consecutive years [45].

The present study showed that there was a significant increase in serum urea and creatinine in infected animals than healthy ones. The results agreed with El-Dessouky and El-Masry [15] who revealed that there were significant increases in serum urea and creatinine levels. While, Osman and Sadiek [18] detected a significant increase in serum urea levels with insignificant increase in serum creatinine. The noticeable increase in serum blood urea level may be attributed to deficient renal blood flow and reduced urine formation in calves with fluid deficit in an attempt to conserve body fluids [46]. In addition, the significant increase in serum creatinine level in diseased calves can be considered to be due to the high levels of all metabolic waste materials which include creatinine resulted from reduced amount of urine in an attempt to conserve body fluids [47].

In conclusion, *Cryptosporidium* is a parasite which has the ability to induce diarrhea in calves and gradually decrease with age, the adults may harbor the parasite without any symptoms. In addition, the disease is greatly affected by managemental system and has a relationship with seasonal variations and has an adverse effect on biochemical parameters of infected calves.

REFERENCES

1. Smith, H.V., S.M. Caccio, N. Cook, R.A. Nichols and A. Tnbait, 2007. *Cryptosporidium* and *Giardia* as foodborne zoonoses. Vet. Parasitol., 149: 29-40.
2. Karanis, P., C. Kourenti and H. Smith, 2007. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J. Water Health, 5: 1-38.

3. Mallinath, R.H.K., P.G. Chikkachowdappa, A.K.J. Gowda and P.E. D'Souza, 2009. Studies on the prevalence of cryptosporidiosis in bovines in organized dairy farms in and around Bangalore, South India. Vet. Arhiv, 79(5): 461-470.
4. Klein, P., T. Kleinová, Z. Volek and J. Šimùne, 2008. Effect of *Cryptosporidium parvum* infection on the absorptive capacity and paracellular permeability of the small intestine in neonatal calves. Vet. Parasitol., 152: 53-59.
5. Ozdal, N., P. Tanritanir, Y. Göz, S. Deger and S. Kozat, 2009. Parasitic protozoans (*Eimeria, Giardia* and *Cryptosporidium*) in lambs with diarrhoea in the Van Province (Turkey). Bull. Vet. Inst. Pulawy, 53: 47-51.
6. Olson, M.E., R.M. O'Handley, B.J. Ralston, T.A. McAllister and R.C. Thompson, 2004. Update on *Cryptosporidium* and *Giardia* infections in cattle. Trends Parasitol., 20: 185-191.
7. Fayer, R., M. Santin, J.M. Trout and E. Greiner, 2006. Prevalence of species and genotypes of *Cryptosporidium* found in 1-2- years old dairy cattle on farms in eastern United States. Vet. Parasitol., 135: 105-112.
8. Feng, Y., Y. Ortega, G. He, P. Das, M. Xu, X. Zhang, R. Fayer, W. Gatei, V. Cama and L. Xiao, 2007. Wide geographic distribution of *Cryptosporidium bovis* and the deer-like genotype in bovines. Vet. Parasitol., 144: 1-9.
9. Santín, M., J.M. Trout and R. Fayer, 2008. A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. Vet. Parasitol., 155: 15-23.
10. Sanders, J., S. Putnam, P. Gould, J. Kolisnyk, N. Merced, V. Barthel, P. Rozmajzl, H. Shaheen, S. Fouad and R. Frenck, 2005. Diarrheal illness among deployed U.S. military personnel during Operation Bright Star 2001-Egypt.Diagn.Microbiol. Infect. Dis., 52(2): 85-90.
11. El-Sherbini, G.T. and K.A. Mohammad, 2006. Zoonotic cryptosporidiosis in man and animal in farms, Giza Governorate, Egypt.J. Egypt. Soc. Parasitol., 36(2): 49-58.
12. El-Khodery, S. and S. Osman, 2008. Cryptosporidiosis in buffalo calves (*Bubalus bubalis*): prevalence and potential risk factors. Trop. Anim. Health Prod., 40(6): 419-426.
13. Youssef, F., I. Adib, M. Riddle and C. Schlett, 2008. A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol., 38(1): 9-28.
14. Abd-El-Wahed, M.M., 1999. *Cryptosporidium* infection among sheep in Qalubia Governorate, Egypt. J. Egypt. Soc. Parasitol., 29: 113-118.
15. El-Dessouky, S.A. and N.M. El-Masry, 2005. Effect of *Cryptosporidium parvum* infection on the haematology and blood chemistry of buffalo calves with special reference to the prevalence of infection in adult buffaloes. Assiut Vet. Med. J., 51(105): 108-123.
16. Abdel-Messih, I.A., T.F. Wierzba, R. Abu-Elyazeed, A.F. Ibrahim, S.F. Ahmed, K. Kamal and R. Frenck, 2005. Diarrhea associated with *Cryptosporidium parvum* among young children of the Nile River Delta in Egypt. Journal of tropical pediatrics, 51(3): 154-159.
17. El-Mohamady, H., I.A. Abdel-Messih, F.G. Youssef, M. Said, H. Farag, H.I. Shaheen, D.M. Rockabrand, S.B. Lubyb, R. Hajjeha, J.W. Sandersa, M.R. Montevillea, J.D. Klenaa and R.W. Frencka, 2006. Enteric pathogens associated with diarrhea in children in Fayoum, Egypt. Diagn.Microbiol. Infect Dis., 56: 1-5.
18. Osman, F.A. and A.H. Sadiek, 2008. Role of Cryptosporidial infection as a pathogen of neonatal calf diarrhea in Assiut Governorate. 13th Cong. Fac. Vet. Med. Assiut Univ., pp: 23-25.
19. Shoukry, N.M., H.A. Dawoud and F.M. Haridy, 2009. Studies on zoonotic cryptosporidiosis parvum in Ismailia Governorate, Egypt.J. Egypt. Soc. Parasitol., 39(2): 479-488.
20. Henriksen, S.A. and J.F. Pohlenz, 1981. Staining of Cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand., 22: 594-596.
21. Fayer, R. and L. Xiao, 2008. *Cryptosporidium* and Cryptosporidiosis. 2nd ed., Boca Raton: CRC Press, Taylor and Francis Group, Boca Raton, FL, USA.
22. Fawcett, J.K. and J.E. Scott, 1960.A rapid and precise method for the determination of urea. J. Clin. Path., 13: 156-159.
23. Bartels, H., M. Böhmer and C. Heierli, 1972. Serum creatinine determination without protein precipitation. Clin. Chim. Acta., 37: 193-197.
24. El-Sherif, A.M., M.A. Abdel-Gawad, H.S. Lotfy and K.A.M. Shokier, 2000. Impact of gastrointestinal nematodes and some enteric protozoal affections on the health of buffalo calves. Assiut. Vet. Med. J., 43(86): 260-270.

25. Amer, S., H. Honma, M. Ikarashi, C. Tada, Y. Fukuda, Y. Suyama and Y. Nakai, 2010. *Cryptosporidium* genotypes and subtypes in dairy calves in Egypt. Vet. Parasitol., 169: 382-386.
26. Ramirez, N.E., L.A. Ward and S.A. Sreevatsan, 2004. Review of biology and epidemiology of cryptosporidiosis in humans and animals. Microbiol.Infect., 6: 773-785.
27. Silverlås, C., U. Emanuelson, K. de Verdier and C. Bjorkman, 2009. Prevalence and associated management factors of *Cryptosporidium* shedding in 50 Swedish dairy herds. Prev. Vet. Med., 90: 242-253.
28. Kvac, M., M. Kouba and J. Výtovec, 2006. Age-related and housing dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. Vet. Parasitol., 137: 202-209.
29. Duranti, A., S.M. Caccio, E. Pozio, A. di Egidio, M. de Curtis, A. Battisti and P. Scaramozzino, 2009. Risk factors associated with *Cryptosporidium parvum* infection in cattle. Zoonoses Public Health, 56: 176-182.
30. Yin, J.H., Z.Y. Yuan, H.X. Cai, Y.J. Shen, Y.Y. Jiang, J. Zhang, Y.J. Wang and J.P. Cao, 2013. Age-related infection with *Cryptosporidium* species and genotype in pigs in China.Biomed. Environ. Sci., 26(6): 492-495.
31. Fayer, R., M. Santín and D. Dargatz, 2010. Species of *Cryptosporidium* detected in weaned cattle on cow-calf operations in the United States. Vet. Parasitol., 170(3-4): 187-192.
32. Khan, S.M., C. Debnath, A.K. Pramanik, L. Xiao, T. Nozaki and S. Ganguly, 2010. Molecular characterization and assessment of zoonotic transmission of *Cryptosporidium* from dairy cattle in West Bengal, India. Vet. Parasitol, 171: 41-47.
33. Muhid, A., I. Robertson, J. Ng and U. Ryan, 2011. Prevalence of and management factors contributing to *Cryptosporidium* sp. infection in pre-weaned and post-weaned calves in Johor, Malaysia Exp. Parasitol., 127: 534-538.
34. Maikaia, B.V., J.U. Umohb, J.K.P. Kwagab, I.A. Lawalc, V.A. Maikaid, V. Camae and L. Xiao, 2011. Molecular characterization of *Cryptosporidium* spp. in native breeds of cattle in Kaduna State, Nigeria Vet. Parasitol., 178: 241-245.
35. Hassanain, M.A., Fathia A.M. Khalil, K.A. Abd El-Razik and R.M. Shaapan, 2011. Prevalence and molecular discrimination of *Cryptosporidium parvum* in calves in Behira Province, Egypt. Res. J. Parasitol., 6: 101-108.
36. Benjamin, N., C. Uchechukwu, D. Ikechukwu, A. Oliver and N. Muodebe, 2013. Cryptosporidiosis among children in some rural parts of Imo state, Nigeria. J. Public Health and epidemiology, 5(11): 440-444.
37. Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: An update. Exp. Parasitol., 124: 80-89.
38. Santin, M. and J.M. Trout, 2008. Companion animals. *Cryptosporidium* and Cryptosporidiosis, 437-450.33-Fisher, E.W., 1965.Death in neonatal calf diarrhea. Br. Vet. J., 121: 132-138.
39. Singh, B.B., R. Sharma, H. Kumar, H.S. Banga, R.S. Aulakh, J.P.S. Gill and J.K. Sharma, 2006. Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. Vet. Parasitol., 140: 162-165.
40. Mehdiazami, M., 2007. Prevalence of *Cryptosporidium* infection in cattle in Isfahan, Iran. J. Euk. Microbiol., 54: 100-102.
41. Lin, Q., W. Ren, M. Gao, X. Wang, M. Cong, W. Cheng, B. Hu, H. Li, S. Yu and G. Zhao, 2012. Prevalence of the intestinal parasite infection in cattle in Shaanxi province, northwestern China. African J. Microbiol. Res., 6(33): 6252-6256.
42. Khalil, F.A.M., 1993. Studies on *Cryptosporidium* infection in calves M.V.Sc. Thesis Fac. Vet. Med., Cairo University.
43. Lefay, D., M. Naciri, P. Poirier and R. Chermette, 2000. Prevalence of *Cryptosporidium* infection in calves in France. Vet. Parasitol., 89: 1-9.
44. Hamnes, I.S., B. Gjerde and L. Robertson, 2006. Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway.Vet. Parasitol., 140(3-4): 204-416.
45. Castro-Hermida, J.A., I. García-Presedo, A. Almeida, M. González-Warleta, J.M. Da Costa and M. Mezo, 2009. Detection of *Cryptosporidium* spp. and *Giardia duodenalis* in surface water: A health risk for humans and animals. Water Res., 43(17): 4133-4142.
46. Fisher, E.W., 1965. Death in neonatal calf diarrhea. Br. Vet. J., 121: 132-138.
47. Walt, J.G., 1965. The use of fluid replacement in the treatment of neonatal diseases in calves. Vet. Rec., 77: 1474.