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ORIGINAL PAPER

Cryptosporidiosis in buffalo calves (*Bubalus bubalis*): Prevalence and potential risk factors

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Abstract The objective of the present study was to describe the prevalence and risk factors associated with cryptosporidiosis in buffalo calves in Middle Egypt. During one year, 458 fecal samples were collected from buffalo calves less than 3 month age in 55 small scale herds and examined for the presence of *Cryptosporidium* oocysts. Data describing age, gender, season, and herd management practices were gathered to assess potential risk factors. Fecal examination showed that 14.19% of the examined calves were positive for *Cryptosporidium* spp. Calves at 1–15 days were at the highest risk ($P<0.001$), and a significant relationship between season and infection ($P<0.05$) was recorded. A significant association between infection and hygiene ($P<0.001$), type of floor ($P<0.01$) and source of water ($P<0.01$) was also recorded. Statistical analysis concerning the clinical signs and fecal characteristics revealed a significant association with fecal consistency ($P<0.001$), presence of blood ($P<0.01$) and mucous ($P<0.01$). Moreover, a significant association was found between infection and the desire for suckling ($P<0.05$) and tenesmus ($P<0.05$). The results of the present study demonstrated the strong relation between infections by *Cryptosporidium* spp. and diarrhea in buffalo calves.

Keywords Cryptosporidiosis · Buffalo calves · Prevalence · Risk factors · Egypt

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Introduction

Protozoans belonging to the genus *Cryptosporidium* are frequent agents of gastrointestinal infection in humans, domestic animals, and other vertebrates. Three species of *Cryptosporidium* have been associated with infection in cattle. Two small-type oocysts, *C. parvum* and *Cryptosporidium bovis* (Fayer et al. 2005), infect the small intestine. However, the larger type, *Cryptosporidium andersoni* that infects the abomasum has been implicated as a cause of reduced milk production in diary cattle (Lindsay et al. 2000).

Cryptosporidium parvum is the most frequently detected pathogen in calves less than 3 weeks age (Moore and Zeman 1991; de la Fuente et al. 1999), where it considered being one of the main common causes of diarrhoea at this age (Koudela and Bokova 1997). However, cryptosporidiosis should not only be considered from the perspective of animal health and production; its zoonotic character and the possibility that animals may act as a source of infection to humans, via foodstuff and water, should also be considered. Although the infection leads to few deaths, serious economic losses can occur due to costs involved in the treatment (de Graaf et al. 1999). Single infection with *C. parvum* is usually present in diarrheic calves; however, mixed infection with other pathogens exaggerates the problem (Vanopdenbosch et al. 1979).

Treatment of cryptosporidiosis is not effective in cattle. So, control measures are mainly based on preventive measures rather than treatment (Woods et al. 1996). However, halofuginone lactate was found to have anticryptosporidial effect (Jarvie et al. 2005; Klein 2007). Furthermore, *Cryptosporidium* oocysts are resistant to the commonly used disinfectant at recommended concentrations (Campbell et al. 1982). It is necessary to find management approaches to minimize the risk of infection with cryptosporidiosis. Cryptosporidiosis in water buffaloes (*Bubalus bubalis*) received great interest in different localities of the world (Galiero et al. 1994; Dubey et al. 1992; Rinaldi et al. 2007). In Egypt, studies limited to the prevalence and zoonotic aspects of the disease were done (Iskander et al. 1987; El-Sherif et al. 2000; El-Dessouky and El-Masry 2005; El-Sherbini and Mohammad 2006). The prevalence of cryptosporidiosis was also mentioned in African buffaloes (*Syncerus caffer*) among wildlife animals in Tanzania (Mtambo et al. 1997). However, the risk factors concerned with cryptosporidiosis in buffalo calves have not been described. Consequently, the objective of the present study was to determine the prevalence and determinants (risk factors) of cryptosporidiosis in buffalo calves in Middle Egypt.

Materials and methods

Calves and data collection

During the period from November, 2005 to October, 2006, 458 Buffalo calves (299 diarrheic and 159 non-diarrheic) under 3 month of age in 55 small scale herds (5–40 animals) were investigated for detection of *Cryptosporidium* spp. infections in the Middle Egypt (Dakahlia and Kafr El-Sheikh governorates). The animal's identification, age, gender, and number of animals per herd were recorded. Clinical examination of each calf was performed and the clinical parameters related to diarrhea such as rectal temperature, hydration status, suckling desire, fecal characters and presence of tenesmus were also recorded. A questionnaire was done about the housing conditions, type of flooring, hygienic measures, source of drinking water, presence of other animals and introduction of new calves to the farm. Furthermore, there was a series of questions about the management and raising of the newborn calves.

Sampling and samples processing

Fecal samples were obtained directly from the rectum in a separate clean labeled container and examined macroscopically for consistency and presence of blood and/or mucous. Fecal smears were prepared and stained using modified Ziehl-Neelsen stain (Henricksen and Pohlenz 1981). Intensity of infection was detected by counting the cryptosporidial oocysts (1000x magnification field) according to Anderson and Bulgin (1981); mild degree (1–5 oocysts/field), moderate degree (6–20 oocysts/field), and severe degree (more than 20 oocysts/field).

Statistical analysis

All data analyses were carried out using statistical software program (SPSS for Windows, Version 15.0, USA). Association between the occurrence of *Cryptosporidium* spp. infection and the potential risk factors were studied using logistic regression. At first step an univariate logistic regression was carried out. In this method, the dependent dichotomous variable was the status of the calves (infected or non-infected). However, the independent variables were the hypothesized risk factors. Variables with significance at $P < 0.1$ were selected for further multivariate logistic regression model. Hosmer and Lemeshow's goodness of fit statistic test greater than 0.05 was used to imply that the model's estimates fit the data at an acceptable level in multivariate analysis. The results were each expressed as P value and odds ratio (OR) with a 95% confidence interval (CI 95%). A chi-square (χ^2) analysis test was used to study the possible association between the infection and the variables of clinical findings and fecal characters. For those variables with more than two categories, Chi-square of linear trend

Table 1 Final logistic regression model for positive risk factors associated with cryptosporidiosis in buffalo calves

Variable	β	SE	P	Odds	CI
Age	−0.656	0.139	0.000	0.710	0.441–1.11
Season	−0.377	0.151	0.017	0.886	0.541–1.15
Hygiene	1.891	0.499	0.000	6.625	2.489–17.631
Floor type	1.129	0.426	0.008	2.220	1.340–7.132
Water source	−1.253	0.380	0.001	0.268	0.246–0.913
Constant	−1.516	0.440	0.037	0.306	-

β: Regression coefficient

SE: standard error

CI: confidence interval

was used and the results were considered to be significant at $P<0.05$.

Results

Out of the examined 458 buffalo calves, *Cryptosporidium* oocysts were detected in 65 (14.19%) calves. After the construction of a multivariable model, Hosmer and Lemeshow's goodness of fit test statistic revealed that the model adequately fit the data ($\chi^2=9.584$; $P=0.295$). As shown in Table 1, five risk factors were found to affect the prevalence of *Cryptosporidium* spp. infection. Animal age significantly affected the prevalence ($P<0.001$; OR: 0.71; CI 95%:0.441 −1.11); 1–15 day-old-calves were the most affected (61.40%); however calves at 60–90 days of age didn't shed oocysts (Fig. 1). Season was also found to affect the prevalence of infection ($P<0.05$; OR: 0.888; CI 95%:0.541–1.15). Winter recorded the highest infection rate (49.12%); however, summer recorded the lowest rate (Fig. 2). In contrast, gender showed no significant effect on the prevalence of cryptosporidium infection ($P=0.159$; OR: 0.415; CI 95%:0.12–1.412).

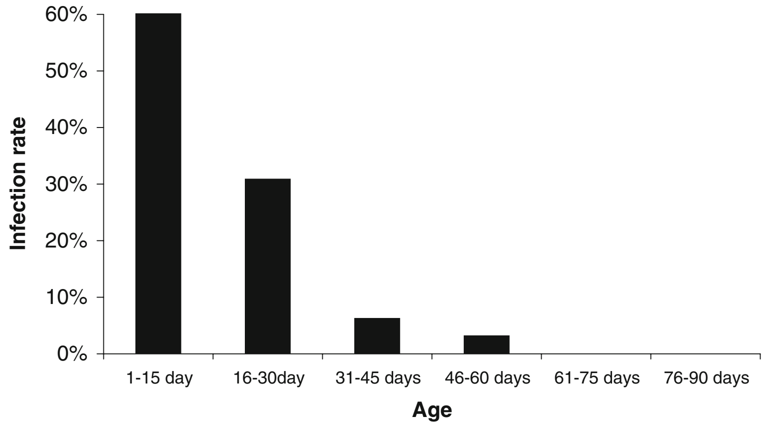
Analysis the type of the floor as a risk factor showed a significant differences in the prevalence of cryptosporidiosis on farms with earth flooring and cement flooring ($P<0.01$; OR: 2.22; CI 95%:1.340–7.32). Thus, the risk of infection was higher in calves housed in pens with concrete floors than in those with earth floors. The frequency of cleaning (daily or weekly) also had a significant effect on the risk of infection. Calves on herds where pens were cleaned once weekly had higher prevalence than those pens cleaned daily ($P<0.001$; OR: 6.625; CI 95%:2.489–17.631).

Water source was found to affect the prevalence of the infection ($P=0.001$; OR: 0.268; CI 95%:0.246–0.913), calves drink from the wells or underground water had higher infection than those were drinking from the tap water.

Separation from the mother, presence of other animals, housing, herd size and adding of new calves to the herd were found to have no significant effect on the disease prevalence. The remaining characteristics as animal housed single or mixed, treatment of excreta, and use of disinfectant were not subjected to statistical analysis because they were similar in all herds.

In regard to clinical findings, there was a significant association between the infection and diarrhea ($P<0.001$; linear trend, $P<0.001$). Thus, 53 (81.53%) of the parasitized calves had diarrhea compared with 12 (18.46%) with normal fecal consistency. Thirty two calves had soft pasty feces, whereas 21 were showing watery diarrhea. Overall results showed that diarrheic calves had higher rate of infection (17.72%) than non-diarrheic ones (7.54%). There was a significant association ($P<0.01$) between *Cryptosporidium* infection and the presence of mucus in the feces; 36 (55.38%) of the infected calves had mucus in their feces. Also, there was a relationship between the presence of *Cryptosporidium* oocysts and the existence of blood in the feces ($P<0.01$), 63.07% of the infected calves had blood in their feces, versus 36.93% calves. Moreover, there was significant association between the severity of infection and presence of blood, mucous or both in the feces (χ^2, $P=0.002$; linear trend test, $P=0.001$), thus 21 cases of severely infected calves had combined mucous and blood versus five of mild infection and 10 of moderate infection. There was a significant association between the severity of infection and the fecal consistency ($P<0.001$; linear trend test, $P<0.001$). Thus, 91.30% of severely infected calves has watery diarrhea and 100% of moderately infected cases had soft pasty feces, and only 12 mildly infected calves with normal feces shed oocysts. No significant association was recorded between *cryptosporidium* infection and presence of fever and dehydration ($P=0.275$ and $P=0.410$, respectively); however, significant association was recorded between infection and the desire for suckling ($P<0.05$). Thus, 34 (52.30%) of infected calves stopped suckling compared with 31 (47.69%) had normal suckling. Furthermore, a significant association between the infection and tenesmus

Fig. 1 Age distribution of cryptosporidiosis in buffalo calves (n=65)



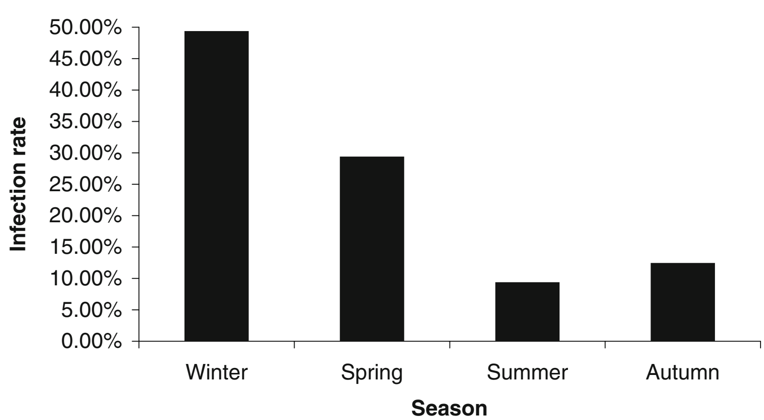
(*P*<0.05); 37 (56.69%) of the affected calves were suffering from such sign.

Discussion

The objective of the present study was to describe the prevalence and risk factors of *Cryptosporidium* infection in buffalo calves. To our knowledge, there are no published reports dealing with risk factors and sufficient causes associated with cryptosporidiosis in buffalo calves. The prevalence of infection was 14.19%, which was found less than that recorded in water buffaloes (Iskander et al. 1987; Galiero et al. 1994) as well as wild buffalos (Mtambo et al. 1997). The variation between the different prevalence rates of the disease may be attributed to the system of rearing and management in addition to the level of hygienic measures applied. Moreover, it may be related also to the presence of many sufficient causes and a number of contributing factors. It was also found that the prevalence in buffalo calves was less than that described in bovine calves in different localities of the world (Castro-Hermida et al. 2002; Santín et al. 2004). In the present study, all of the calves were not separated from their mothers directly after birth and had taken their colostrums directly from their dams. It is suggested that natural feeding and delayed separation could played a role in the low prevalence compared with bovine calves. Quigley et al. (1994) and Mohammed et al. (1999) observed a decrease in the risk of infection in calves which were separated from their mothers after only a few hours and fed manually.

In the present results, the final logistic regression model made it possible to identify five factors that were significantly associated with the risk of infection with *Cryptosporidium* spp. in buffalo calves including age, season, hygiene, floor type and water supply (Table 1). The prevalence of infection by *Cryptosporidium* spp.

Fig. 2 Seasonal distribution of cryptosporidiosis in buffalo calves (n=65)



varied considerably with the age of the calves, although there was a controversy about the prevalence of cryptosporidiosis in relation to the age. The highest prevalence was found in calves at 1–15 days-old (60%) with progressive decrease until two month of age. Calves at 61–90-days of age recorded no infection. This finding coincided with that recorded by Wade et al. (2000) and de la Fuente et al. (1999). On contrary, cryptosporidiosis was recorded in older bovine calves (Maddox-Hyttel et al. 2006). Fayer et al. (1998) and Castro-Hermida et al. (2002) stated that the prevalence of bovine cryptosporidiosis is underestimated because of the low number of samples taken during the pre-weaning period. Villacorta et al. (1991) recorded prevalence of 93.0% in 3–6-day-old calves selected randomly and examined twice weekly for one month. In contrast, when only one or two fecal samples per calf were examined during the pre-weaning period, less than 30.0% of the calves were found to have oocysts in their feces (Garber et al. 1994; Maldonado et al. 1998).

A significant association between the season and the prevalence of *Cryptosporidium* spp. infection was recorded in this study. Winter recorded the highest prevalence (49.22%), while summer recorded the lowest (9.23%). It is suggested that the prevalence of infection by *Cryptosporidium* spp. is not only related to the presence of calves at risk but also related to the presence of the suitable climatic condition for viability and spread of the parasite. In winter, the temperature in our locality is suitable for viability and survival of *Cryptosporidium* oocysts. Similar findings were recorded by Tzipori (1983) and Lefay et al. (2000). These results were supported by Anderson (1986) who found that warm temperature of 18 to 29°C, had been partially responsible for loss of the infectivity. However, Garber et al. (1994) attributed the high prevalence of cryptosporidiosis in winter to presence of large number of calves at risk a result of concentration of calving in winter months. On contrary, in California, Atwill et al. (1999) found that calves were at high risk of contracting infection by *Cryptosporidium* spp. during May month and they attributed this to the greatest contact with the source of infection, lowering animal resistance or environmental conditions that might favor transmission of the infection during this month. Contrary to all recorded results, absence of seasonality in the presence of *Cryptosporidium* infection has been described (Wade et al. 2000; Castro-Hermida et al. 2002). It was suggested that seasonal effects can only be correctly evaluated when the study is repeated over several consecutive years (Castro-Hermida et al. 2002).

Concerning the role of floor type and hygiene as risk factors, Prevalence was found to increase when animals housed in places with cement floors rather than earth floors ($P<0.01$). In houses with cement floor there was no efficient cleaning or using of disinfectants, which could help to retain dirties and moisture. Furthermore, mixing of the animals with each other may help in contraction and spread of infection. On contrary, Castro-Hermida et al. (2002) found that cement floor had lower risk than straw/earth floor due to daily cleaning using water with pressure hose. The use of straw and deep litter seemed to confer protection against Cryptosporidium infection or at least excretion (Maddox-Hyttel et al. 2006) . They attributed this finding to the microclimatic factors governing oocyst survival. It was also found that calves in herds with cleaning daily had low tendency to have infection than that cleaned weekly ($P<0.001$). In bovine calves, a similar finding was also recorded by Castro-Hermida et al. (2002). Moreover, there was tendency for the risk of infection to decrease when animals were housed individually in pens previously disinfected with bleach or lime (Garber et al. 1994; Quigley et al. 1994; Mohammed et al. 1999).

Water supply was found to affect the prevalence of the cryptosporidiosis; calves consumed water from wells showed higher prevalence than those consumed tap water. This may be attributed to pollution of underground water with drainage. *Cryptosporidium* oocysts have been demonstrated in run-off from

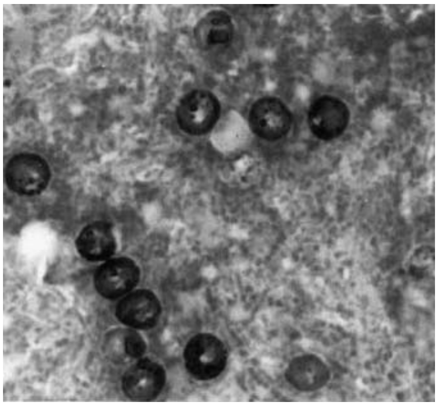


Fig. 3 *Cryptosporidium* oocysts identified form buffalo calves (Ziehl-Neelsen X1000)

agricultural areas, and outbreaks at least of cryptosporidiosis in humans have been ascribed contamination of drinking and bathing water with such effluents (Fayer 2004). On the other hand, Castro-Hermida et al. (2002) didn't record obvious relationship between the source of water and the prevalence of cryptosporidiosis in bovine calves.

Concerning the herd size, there was no significant effect of the herd size on the occurrence of *Cryptosporidium* spp. infections. This finding may be attributed to small herd size. However, Castro-Hermida et al. (2002) fond that the smaller numbers of adult cattle in the farm the greater prevalence of cryptosporidiosis. On contrary, it was stated that the higher the density of animals, the greater the number of infected calves and consequently higher prevalence (Garber et al. 1994; Quigley et al. 1994; Mohammed et al. 1999).

In regard to clinical signs, as previously mentioned, the main clinical sign of cryptosporidiosis is diarrhea. Other secondary signs such as tensmus and decreased desire for suckling were recorded. The infection rate in diarrheic calves was 17.72%, whereas in non-diarrheic calves was 7.54%. This result came in accordance with that carried out in cattle by Atwill et al. (1999) and Castro-Hermida et al. (2002). Coexistence of *C. parvum*, rotavirus, coronavirus and *Salmonella*, among others, in calves of less than one month of age, with diarrhea was recorded (de la Fuente et al. 1999). Blood, mucous were found to be associated with *Cryptosporidium* spp. infections. On contrary, infection was found not associated with bloody diarrhea due to the very superficial location of the parasite (Heine et al. 1984; Aurich et al. 1990; Blewett and Angus 1994).

Although the infection leads to few deaths, serious economic losses can occur because of problems associated with the resulting diarrhea and dehydration, weight loss and slow growth (Sanford and Josephson 1982). In this study, there was no deaths among infected calves, however it can't be ruled out that death may occur due cryptosporidiosis. Unfortunately, we did not estimate the loss of body weight associated with *Cryptosporidium* infection, suggesting that other infections may coexist and the effects were not due to cryptosporidiosis only.

The identified oocysts from both diarrheic and non diarrheic calves had the microscopic features of *C. parvum*, i.e. oocysts were spherical, 4–5 μm diameter and have the acid fast stain with modified Ziehl-Neelsen (Fig. 3). However, *C. bovis* was found also to infect cattle calves and could not be differentiated by the traditional methods (Santín et al. 2004). *C. parvum* was only found to be predominant in unweaned calves, whereas *C. bovis* was detected in weaned cattle calves (Santín et al. 2004; Fayer et al. 2005; Starkey et al. 2006). *C. andersoni* infects the abomasums of juvenile and mature cattle and this infection has been identified as a cause of reduced milk production. Moreover, in a study, Gómez-Couso et al. (2005) has been identified a *Cryptosporidium* spp. from buffalo heifers without diarrhea using the molecular characterization. In conclusion, similar to cattle calves, the results of the present investigation demonstrate the relation between *Cryptosporidium* infection and the occurrence of diarrhea in buffalo calves. Further molecular biological study needs to be done to recognize the *Cryptosporidium* spp. specific to the buffalo calves in Egypt.

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