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Dairy Farm Management Practices as Risk Factors Linked to *Cryptosporidium* spp. Infection in Dairy Calves

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ABSTRACT

Background: Cryptosporidiosis is the disease of zoonotic importance and is considered to be third major cause of diarrhoeal disease of calves worldwide causing significant economic losses. The present study aimed to link *Cryptosporidium* spp. infections in dairy calves with the management practices as a major risk factor.

Methods: Total 401 faecal samples from dairy calves were collected from eight large (100-1000 animals) and eight medium size (30-100 animals) dairy farms located in Jabalpur (Madhya Pradesh, India). *Cryptosporidium* oocysts were identified in faeces by modified Ziehl-Neelsen staining (mZN) and formal ether concentration techniques.

Result: The prevalence of *Cryptosporidium* spp. was 28.7% in dairy calves. A higher prevalence and risk of cryptosporidiosis was associated with large farms (24.9% vs. 3.74%, OR=1.96), intensive farming system (27.7% vs. 0.99%, OR=1.86), drinking ground water (27.7% vs. 0.99%, OR=1.86), dirty farms (16.7% vs. 11.6%, OR=1.90), presence of other animals at farm (15.9% vs. 12.7%, OR=1.36), absence of deworming (14.7% vs.13.9%, OR=5.09) and diarrhoea treatment practice (16.2% vs. 12.5%, OR=1.07). Multi-animal calving area, dirty calving pen and cemented floor were also identified as risk factors. Practices like feeding colostrum within one hour of birth, @ 10% b.wt./day, weaning the calves within 24 hrs and using detergent to wash feeding utensils decreased the risk of infection from 1.4 to 2 times. Calves kept confined with their dam had greater prevalence and risk of infection (17.2% vs. 11.5%, OR=5.20). The findings highlight that proactive managemental practices are beneficial for decreasing the environmental contamination and improving calf health.

Key words: *Cryptosporidium* spp, Dairy calves, Management practices, Risk factor.

INTRODUCTION

Cryptosporidiosis (Apicomplexan protozoan) is of global importance, being an emerging zoonotic disease. Young calves are primarily affected with *Cryptosporidium* spp. on dairy farms. It incurs heavy economical losses in terms of increased labor, medicinal costs and losses in terms of calf deaths (de Graaf *et al*., 1999). A mortality rate of 35.5 per cent in calves of 0 to 30 days has been reported from Punjab (Singh *et al*., 2006). Feco- oral route is the main mode of infection, as substantial numbers of oocysts are shed by infected calves in their feces (Nydam *et al*., 2001). Different researches have examined the affiliation between the episodes of *C. parvum* on dairy farms and farm management practices, variations may be seen depending on different geographical regions. The present study aimed to determine the management practices as risk factors linked to *Cryptosporidium* spp. infection in dairy calves.

MATERIALS AND METHODS

Study area

The work was undertaken in the dairy farms located in Jabalpur, Madhya Pradesh, India. It is situated at 23.1° latitude and 79.57° longitude at 410.87 MSL (meters above sea level) in southern part of agro-climatic zone *viz*., Kymore plateau and Satpura hills and has an average rainfall of 1241 mm.

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Samples collection

A total of 401 faecal samples from dairy calves were collected randomly from eight large (100-1000 animals) and

eight medium size (30-100 animals) dairy farms located in Jabalpur (Madhya Pradesh). Faecal sample from rectum of each animal were collected in clean, sterile plastic bags or stool container using separate gloves. The collected samples were directly transported to the laboratory for processing. The samples were kept in 2.5% potassium dichromate solution at 4°C and then brought to laboratory.

Examination of faecal samples for cryptosporidiosis

The collected faecal samples were stained by modified Ziehl-Neelsen stain (mZN) (Henricksen and Pohlenz, 1981) for identification of *Cryptosporidium* spp. oocyst. Formal ether sedimentation technique for detecting oocysts of *Cryptosporidium* spp. was used, as recommended for the concentration of parasites in faeces (Ash *et al*., 1994).

Questionnaire based survey to identify managemental risk factors associated with cryptosporidiosis in dairy calves

A questionnaire was designed and survey was conducted on general and calf management practices to correlate prevalence of *Cryptosporidium* spp. infection in dairy calves.

Statistical analysis of risk factor

Managemental practices as risk factors associated with *Cryptosporidium* spp. infection in dairy calves was analyzed through independent Chi-square test and the odds ratio was determined at 95% CI using online program (http://statpages.org/ctab2x2.html).

RESULTS AND DISCUSSION

Association of managemental factors of discrete nature with prevalence of *Cryptosporidium* spp. in dairy calves and their analysis is indicated in Table 1.

General farm management practices

In dairy calves prevalence of *Cryptosporidium* spp. infection as per farm size was observed to be 24.9 per cent in large farms (100-1000 animals) and 3.74 per cent in medium size (30-100 animals) farms. Risk ratio suggested that the calves of large dairy farms were at 1.96 time's higher risk for cryptosporidiosis than those reared in medium sized farm. Several scientists have traced an interrelation between sizeable farms and the risk of infection (Garber *et al*., 1994; Quigley *et al*., 1994 and Mohammed *et al*., 1999). Garber *et al*. (1994) found higher prevalence in large (>200 dairy cows) and medium (100-200 dairy cows) herds than in small (<100 dairy cows) herds. Large herds were significantly more likely to have *Cryptosporidium* infected calves than were small herds. It is possible that large herds may have a greater density of animals with heavier pathogen load. As a consequence of greater density it may not be possible to prevent continual accumulation of occysts in the environment with less time of cleaning. High stocking rate (8-10 animals/ 200 sq.ft) and confinement in close proximity might predispose the susceptible young calves to cryptosporidial infections (Mitchell *et al*., 1997).

The prevalence of cryptosporidiosis in calves was assessed in relation to farming system (intensive and semi-intensive). Higher prevalence was found in calves of intensive (27.7%) than semi-intensive farming system (0.99%). The farms with intensive farming system were at 1.86 time's greater risk than those reared under semi-intensive farming. It may be explained by the fact that intensive indoor rearing is usually associated with poor hygiene and thus may contribute to heavy infection. However, it depends on the previous history of infection and the husbandry management involved.

Animals drinking ground water had higher prevalence (27.7%) of *Cryptosporidium* spp. oocysts in faecal samples as compared to animals drinking tap water supplied by municipality (0.99%). Statistically it was noted that at 95% CI (confidence interval) the value of odd ratio between animals using ground water versus tap water for drinking was 1.86. Therefore, it was inferred that the farms using ground water for drinking were nearly two times more prone for *Cryptosporidium* spp. infection than those using tap water for drinking. Our findings are in accordance with that of El- Khodery and Osman (2008) who found that calves drinking water from wells or underground water were at higher risk of infections than ones consuming tap water (OR=0.268; CI 95%:0.246-0.913) and it may be ascribed to contamination of underground water with sewage. Oocysts of *Cryptosporidium* spp. were found in agricultural run-off water and its outbreak in humans has been assigned to using contaminated water for drinking and bathing (Fayer, 2004). There is a possibility of *C. parvum* oocysts percolating through soil layer in areas where cow manure fertilizer is used.

The prevalence of *Cryptosporidium* spp. was significantly ($p<0.05$) higher (16.7%) in farms where level of cleanliness was found to be 'dirty' (floor of the farms was not clean regularly) as compared to 'clean' farms (floor of the farm was clean regularly) (11.6%). The risk of occurrence of cryptosporidiosis in a dirty farm was nearly twice (OR=1.90) than clean farm. Similar to present finding, Swai and Schoonman (2010) recorded higher risk of exposure and infections with *Cryptosporidium* spp. in calves who slept on dirty floors than the ones on moderate to clean floors (OR=1.82).

The presence of other animals like dog, birds at farm changed the prevalence of cryptosporidiosis in dairy calves. The prevalence was higher (15.9%) in farms with other species like dog, birds were reared alongside as compared to farms with no other species of animal being reared. (12.7%). The risk of infection in farms with other animals reared along (dogs, birds) was also found to be higher (OR=1.36). However, non-significant association was found. These results are in accordance with those reported by Muhid *et al*. (2011) who reported higher prevalence and risk (OR=1.5) in farms where other animals were present.

In the farms where regular deworming of animals with albendazole and fenbendazole was done, the prevalence of *Cryptosporidium* was significantly ($p<0.05$) lower (13.9%) than those who were not dewormed at regular interval (14.7%). The chances of infection were five times higher

Table 1: Management practices as risk factors linked to *Cryptosporidium* spp. infections in dairy calves.

Category	Variables	Per cent of samples positive for *Cryptosporidium* spp. (No. of positive sample)	Risk/Odd ratio	95% confidence interval	*P* value
Farm size					
	Large	24.9 (100)	1.96	1.07-3.60	0.03
	Medium	3.74 (15)	0.51	0.28-0.94	
Management practices					
	Intensive	27.7 (111)	1.86	0.62-5.63	0.27
	Semi-intensive	0.99 (04)	0.54	0.18-1.62	
Source of water					
	Ground water	27.7 (111)	1.86	0.62-5.63	0.27
	Tape water	0.99 (04)	0.54	0.18-1.62	
Level of farm cleanliness					
	Dirty	16.7 (67)	1.90	1.23-2.95	0.00
	Clean	11.9 (48)	0.52	0.34-0.81	
Any other animals on the farm					
	Yes	15.9 (64)	1.36	0.88-2.11	0.16
	No	12.7 (51)	0.73	0.47-1.13	
Deworming of animals					
	Yes	13.9 (56)	0.19	0.12-0.32	0.00
	No	14.7 (59)	5.09	3.16-8.22	
Treat calves with drugs for diarrhoea					
	Yes	12.5 (50)	0.94	0.60-1.45	0.77
	No	16.2 (65)	1.07	0.69-1.65	
Calves born area					
	Multi animal calving area	27.7 (111)	1.86	0.62-5.63	0.27
	Single animal calving area	0.99 (04)	0.54	0.18-1.62	
Calving pens were cleaned before calf birth					
	Yes	13.4 (54)	0.71	0.46-1.09	0.12
	No	15.2 (61)	1.41	0.92-2.18	
Floor type of the calving pen					
	Concrete/cemented	27.7 (111)	1.86	0.62-5.63	0.27
	Earth	0.99 (04)	0.54	0.18-1.62	
Sweep the floor daily					
	Yes	12.5 (50)	0.81	0.53-1.26	0.35
	No	16.2 (65)	1.23	0.79-1.90	
First feeding of colostrum after birth within hour					
	Yes	13.4 (54)	0.71	0.46-1.09	0.12
	No	15.2 (61)	1.41	0.92-2.18	
Separation from dam after calving within 24 hours					
	Yes	0.49 (02)	0.49	0.10-2.26	0.36
	No	28.2 (113)	2.05	0.44-9.49	
Confinement of calves with mother					
	Yes	17.2 (69)	5.20	3.27-8.29	0.00
	No	11.5 (46)	0.19	0.12-0.31	
Colostrum fed to the calves @10% body weight/day					
	Yes	3.2 (13)	0.53	0.28-1.02	0.06
	No	25.4 (102)	1.87	0.98-3.57	
Wash feeding utensils with detergent					
	Yes	2.5 (10)	0.66	0.32-1.38	0.27
	No	26.2 (105)	1.51	0.72-3.16	

Significant at P≤0.05; Non significant at P>0.05.

(OR=5.09) in farms without regular deworming practices. Muhid *et al*. (2011) determined significant association between *Cryptosporidium* spp. infection and anti-helmintic treatment. The calves which were dewormed regularly had less chances of infection (OR=0.28) than the ones dewormed less regularly. A lower (12.5%) prevalence was observed in farms where treatment of diarrhoeic calves was practised as compared to farms where no treatment for diarrhoea was done (16.2%). The risk of infection was almost similar in both type of farms (OR=1.07). Trotz-Williams *et al*. (2008) found significant association between prevalence of *Cryptosporidium* spp. infection and use of prophylactics against calf scour (RR=1.38). Similarly, Muhid *et al.* (2011) reported that the calves that were treated thrice with antibiotic and anti-diarrhoeal drugs in a year had less chances of infection (OR=0.28).

Calf management practices

Calves born in common calving penhad higher prevalence of *Cryptosporidium* spp. oocysts in faecal samples (27.7% vs. 0.99%) as compared to calves born in individual calving pen. The odd ratio indicated nearly two times higher (OR=1.86) chance of infection in prior than latter. The results coincide with that of Garber *et al*. (1994) who observed that farms with multiple cow maternity facilities were more likely to have calves with *Cryptosporidium spp.* infection than those farms with individual facilities.

Post-parturient dams and hutches contaminated with oocysts from previous calf may act as source of *Cryptosporidium* spp. infection to dairy calves (Maldonado-Camargo *et al*., 1998). Cryptosporidia oocyst may also be present in the maternity area from sources other than neonatal calves such as; rodents, cats, human beings or equipment. Therefore, the prevalence of calf cryptosporidiosis in the present study was assessed in relation to the cleaning of calving pens before parturition. It was observed that calves born in dirty calving pens had greater prevalence (15.2% vs. 13.4%) of *Cryptosporidium spp*. Infection. These calves are 1.41 times more prone for the infection than those born in clean calving pens. Garber *et al*. (1994) opined that cryptosporidiosis was not associated with whether or not the maternity area was cleaned. Survival of *Cryptosporidium* oocysts is affected by temperature, ammonia concentration, pH (Reinoso *et al*., 2008) and the floor type is likely to cast an effect on these factors. Thus the impact of floor type of calving pen on *Cryptosporidium* spp. infection was assessed and it was found that the prevalence was higher (27.7%) in calves born on concrete/cemented floor calving pen as compared to earth floor calving pen (0.99%). The risk of infection in calves born in calving pen with concrete/ cemented floor was nearly two times higher (OR=1.86). The findings of our study may be biased as majority of the selected farms were having concrete floors. Similar to our findings, El- Khodery and Osman (2008) observed that type of flooring had a prominent effect on prevalence of cryptosporidiosis on farms and recorded higher risks associated with cemented floors than earthen floors (OR=2.22; CI 95%:1.340-7.32), it could be possible due to retention of moisture and providing favourable conditions for greater prevalence. Maddox-Hyttel *et al*. (2006) suggested that *Cryptosporidium* infections or its excretion in faeces were decreased by the use of straw and deep litter system and this finding was attributed to the microclimatic factors which govern oocyst survival.

The effect of sweeping of floor on the prevalence of calf cryptosporidiosis was also observed. The prevalence was found to be lower (12.5%) in farms where floor was swept daily than where the floor was not swept daily (16.2%). Odd ratio indicated that if sweeping is not done daily at farm, the risk of infection may be higher (OR=1.23). Castro-Hermida *et al*. (2002) observed a significant difference between the effect of monthly and daily cleaning (OR: 1.8; CI 95%: 1.1-3.1); the risk of getting infection was increased by 87.0% in calves whose pens were cleaned monthly than calves whose pens were cleaned daily (OR: 1.9; CI 95%: 1.3-2.9). El- Khodery and Osman (2008) also reported that calves tend to have less chances of getting infection whendaily cleaning of shed is done rather than reared in sheds that were cleaned weekly (OR=6.625; CI 95%: 2.489-17.631).

The prevalence of cryptosporidiosis was lower (13.4%) in farms where calves received colostrum within one hour of birth as compared to farms where calves were not fed colostrum within an hour (15.2%). The odd ratio indicated that the chance of cryptosporidiosis was 1.41 times higher in calves that do not receive colostrum within hour after birth. This is explained by the protecting mechanism of colostral and milk antibodies whereby they block parasitic invasion and immobilize the gut luminal parasitic form, this protects calves from displaying severe clinical signs (Mohammed *et al*., 1999). El-Garhy, (1982) stated that calves were hypogammaglobulinaemic at birth and must obtain immunoglobulins (Ig) during the period of macromolecular transport within the first 24 h of life. So, it is advisable to offer an adequate amount of colostrum in the early period after birth to protect them from the infectious disease during the neonatal period.

The prevalence of cryptosporidiosis was associated with the time of weaning. Analysis revealed that the prevalence was lower in farms where calves were weaned within 24 hours (0.49% vs. 28.2%). Odd ratio indicated that the risk of infection was two times higher (OR=2.05) in calves not separated from dam within 24 hours of calving. Our results coincide with that of Wu *et al*. (2010) who recorded that the prevalence of *Cryptosporidium spp*. on farms where calves were immediately isolated into individual pens was significantly ($p<0.05$) lower (50.0%) than that of the farms which isolated the neonatal calves between second to tenth day after birth (85.0%). This suggested that calves are most susceptible to infection with *Cryptosporidium* spp. shortly after birth and that infection spreads rapidly (McCluskey *et al*., 1995; Quilez *et al*., 1996 and Tortz-Williams *et al*., 2007).

The infection was significantly ($p<0.05$) higher in farms where the calves were confined with their mother (17.2% vs. 11.5%) and such calves were at five times higher (OR=5.20) risk of infection. The findings of present study are in accordance with the findings of Quigley *et al*. (1994) and Mohammed *et al*. (1999) who discerned a lowered infection risks in calves which were separated from their mothers within few hours of birth and fed manually. El-Khodery and Osman (2008) reported non-significant ($p>0.05$) effect of calf separation from the mother on the disease prevalence.

The prevalence of cryptosporidiosis was lower (3.2%) in farms where the calves received colostrum @ 10% body weight/day as compared to their counterparts (25.4%). Risk ratio suggested that the chance of occurrence of *Cryptosporidium* spp. was 1.87 times higher in calves which did not receive colostrum @ 10% body weight/day. The value of adequate colostrum consumption by calves and its effect on calf health is well admitted. Hence, intake of inadequate colostrum (low concentration of IgG) could elevate the infection risks in calves.

Cryptosporidium spp. infection was lower (2.5%) on the farms which used detergent to wash feeding utensils as compared to those where detergent was not used (26.2%). Risk ratio of infection was 1.51 times higher at farms not using detergent. Similar to our results Trotz-Williams *et al*. (2008) found that washing calf feeding equipments with soap or detergent had safeguarding effects and significantly associated with a decreased prevalence.

CONCLUSION

Among general management practices a higher prevalence and risk of cryptosporidiosis was associated with large farms, intensive farming system, ground water as drinking source, dirty farms, presence of other animals at farm, absence of deworming and diarrhoea treatment practice as compared to their counter practices. Among calf management practices, a higher prevalence and risk of cryptosporidiosis was linked to multi-animal calving area, dirty calving pen and concrete/cemented floor in calving pen. Higher prevalence was also observed in farms where the floor was not swept daily, where calves did not received colostrum within one hour of birth, not weaned within 24 hours of calving and kept confined with their mother. The prevalence and risk of *Cryptosporidium* infection were higher at farms where calves did not receive colostrum @ 10% body weight per day and where detergent was not used to wash feeding utensils than in farms where these practices were followed. The findings highlight that proactive management practices are beneficial for decreasing the environmental contamination and improving calf health.

Conflict of interest: None.

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