INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
08–121/ZIP/2009/11–2–221–224
http://www.fspublishers.org

Short Communication

Prevalence of *Cryptosporidium parvum* Infection in Lahore (Pakistan) and its Association with Diarrhea in Dairy Calves

A. NASIR, M. AVAIS[1], M.S. KHAN AND N. AHMAD†
Department of Clinical Medicine and Surgery University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
†Department of Theriogenology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
[1]Corresponding author's e mail: avaismuhammad@yahoo.com

ABSTRACT

The current study was contemplated to find out the prevalence of *Cryptosporidium parvum* in Lahore, Pakistan and its association with diarrhea in dairy calves. For this purpose, 500 faecal samples (n=250 cow calves, n=250 buffalo calves) from different dairy farms and home-bred dairy calves were collected and analyzed by using modified Ziehl-Neelsen staining technique. Overall, 25.6% calves were shedding *C. parvum* with prevalence 27.2% and 24% in cow and buffalo calves, respectively. The pattern of oocyst shedding was negatively correlated with increase in age, highest in the 1-30 days age group and lowest in the age group ranging from 9 months to 1 year and above. It was observed that both shedding and intensity of shedding were significantly higher ($P<0.05$) in diarrheic than in non-diarrheic calves. The findings of this study indicate that *C. parvum* is commonly found among 1-30 days calves and it has strong association with diarrhea in dairy calves in Lahore.

Key Words: Prevalence; *Cryptosporidium parvum*; Diarrhea; Dairy calves

INTRODUCTION

Cryptosporidiosis is an emerging protozoan disease of public health significance. *Cryptosporidium parvum* can cause gastrointestinal illness in a wide variety of mammals, like humans, cattle, sheep, goat, pig and horses through out the world (Fayer, 1997). *C. parvum* has been incriminated as an important cause of diarrhea in neonatal calves (Nydam *et al*., 2001). According to Leek and Fayer (1984) although calves 1–3 weeks old seem to be most susceptible, yet *Cryptosporidium* species has also been found in cattle over two years of age (Henriksen & Krogh, 1985). In immunodeficient human beings, cryptosporidiosis may lead to life-threatening chronic diarrhea and because of the incidence of Acquired Immuno Deficiency Syndrome; the disease poses a significant public health problem in developing countries, where AIDS is endemic. Infection rates are predicted to be highest in developing countries and in children (Fayer, 1997).

The majority of adult cattle can be described as excretors of *C. parvum* oocysts when highly sensitive detection methods are used. Nevertheless, their importance in the transmission of the disease remains questionable, since oocyst excretion by adult cattle was similar in herds with serious problems of cryptosporidial neonatal diarrhea and in those without (Scott *et al.,* 1995). So far, in cattle, no increased output of *C. parvum* oocysts around parturition has been observed (Scott *et al*., 1995; Atwill *et al.,* 1998). Nevertheless, infected newborn calves excrete oocyst numbers of the order of 10^6 to 10^7 g^{-1} of faeces (Current, 1985) and were considered to be a more dangerous source of infection. Infection can rapidly spread from calf to calf when animals are communally housed and over crowded, or from cow to calf via the udders when they are contaminated with infected calf faeces in the lying area of the dams (Angus, 1990). This might explain the association between cryptosporidial diarrhea and the farm type and/or its specific hygienic conditions. Reynolds *et al*. (1986) determined that *Cryptosporidium* infections were more common in single or multiple suckler beef herds and dairy farms with multiple-cow maternity facilities (Garber *et al*., 1994). In the so-called fattening units, where calves are purchased through markets, almost 100% of the animals become infected during transit from the market to the rearing unit or soon after their arrival (Villacorta *et al*., 1991).

This paper describes the prevalence of *C. parvum* in dairy calves and reports its association with diarrhea and the risk factors in this regard.

MATERIALS AND METHODS

This study was conducted at the Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences (UVAS) Lahore. The source of samples was the dairy calves reared by private dairy farmers and home bred dairy calves, located in and around Lahore.

To cite this paper: Nasir, A., M. Avais, M.S. Khan and N. Ahmad, 2009. Prevalence of *Cryptosporidium parvum* infection in Lahore (Pakistan) and its association with diarrhea in dairy calves. *Int. J. Agric. Biol.,* 11: 221–224

A total of 500 faecal samples (n=250 cow calves n=250 buffalo calves) were collected and examined for the presence of *Cryptosporidium parvum* oocysts. The data were recorded on the origin of samples, farm environment (rural, urban or peri-urban, confined or with access to outdoor, if confined, paved or dirt yard, contact with other animals like dogs, cats, rodents, birds), feeding pattern (on milk or fodder or both) of calves; if on milk (suckling from dam or hand feeding), physical condition (emaciated, weak, healthy) of animals and faecal score (1=normal; 2=soft, does not hold form; 3=runny, spreads easily; 4 = devoid of solid matter). Diarrhea was defined as faecal score of 3 or 4 (Larson *et al.,* 1977).

Sample collection and faecal examination. The faecal samples were collected directly from the rectum of each calf using disposable gloves. If an attempt to collect faeces per rectum was unsuccessful and calves were housed individually, a fresh sample was taken from the ground, where available. After collection, the faecal samples were transferred into self sealing polythene bags and preserved in ice before transporting to laboratory. The samples were refrigerated at 4^{o}C in the laboratory till further processing (Young *et al*., 1996) not for more than two days. Each faecal sample was run through oocyst floatation technique using sucrose solution with a specific gravity of 1.18 (Uga *et al.,* 2000). Two grams of faecal material was weighed using an electric balance. Faecal material was added in 3 mL of faecal floatation fluid in 10 mL test tube, which was gradually filled up to 10 mL with continuous mixing to form a positive meniscus. The solution was allowed to stand for 15-20 min. Then, with the help of a pipette or cover slip kept over the filled test tube, a small drop from the top was transferred to clean, grease free and labeled glass slide to make smear. The smears were stained by modified Ziehl-Neelsen (mZN) acid fast staining as described by Casemore (1991).The *C. parvum* was identified by measuring the size using stage micrometry and morphology (Watanabe *et al.,* 2005).

Statistical analysis was evaluated using χ^2 test. Probability (p) of <0.05 was defined as statistically significant.

RESULTS

Prevalence data on *C. parvum* infection in cow and buffalo calves is shown in Table I. Of the total 500 faecal samples tested, 128 were positive showing an overall prevalence of *Cryptosporidium parvum* in dairy calves as 25.6%. Cow calves had high prevalence than the buffalo calves. Amongst the cow calves, the prevalence was higher in crossbred cow calves than Sahiwal cow calves. As far age, the prevalence of infection was significantly higher ($p<0.05$) in 1-30 days old age group of both the cow and buffalo calves followed by 1-3 months, 4-8 months and 9 months-1 year and above age groups. A non-significant difference ($p>0.05$) in prevalence of *C. parvum* infection was observed in male and female cow and buffalo calves. The prevalence of *C. parvum* infection was significantly higher ($p<0.05$) in cow and buffalo calves reared in urban areas and in confined environment. Amongst the healthy, weak and emaciated cow and buffalo calves the prevalence of *C. parvum* infection was non-significant. When compared the prevalence of *C. parvum* infection in cow and buffalo calves on the basis of feeding pattern it was significantly different ($p<0.05$) in milk suckling, fodder eating and both milk suckling and fodder eating calves. The difference in prevalence of *C. parvum* infection was non-significant ($p>0.05$) in cow and buffalo calves kept on paved and dirt yard floor. The calves having some sort of contact with birds/rodents/dogs/cats have had higher prevalence of *C. parvum* infection than the calves having no contact with these animals. Prevalence of *C. parvum* infection in diarrheic and non-diarrheic animals is shown in Table II. It was observed that both shedding and intensity of oocyst shedding were significantly higher ($p<0.05$) in diarrheic than in non-diarrheic animals. The prevalence of infection peaked in young calves between 1-30 days in both diarrheic and non-diarrheic groups. The lowest prevalence of *C. parvum* was observed in cow and buffalo claves of 9 months-1 year and above age group.

DISCUSSION

In the present study the prevalence of *C. parvum* infection in cow and buffalo calves was 27.2% and 24%, respectively. These observations are in conformity to Garber *et al*. (1994), who reported a prevalence of 22.4% in calves in which almost half the calves between 7 and 21 days of age had cryptosporidia in their faecal samples. In the present study the highest prevalence of *C. parvum* infection was reported in 1-30 days age group. Fayer *et al*. (1997) have also reported an oocyst excreting period of 1-12 days for calves. These findings, therefore suggested that oocyst detection rate can vary remarkably depending on the age of the calves. Sturdee *et al*. (2003) reported a prevalence of 52.4% in home-bred and 23.2% in bought-in calves on a low land farm in United Kingdom. A relatively high prevalence in home-bred cow calves may be due to difference in the climatic and managemental conditions as the parasite can survive long in the moist climatic conditions. The results of present study also revealed that *C. parvum* is equally pathogenic for both cow calves (27.2%) and buffalo calves (24%) in all age groups. Singh *et al.* (2006) similarly found that pathogenecity of *C. parvum* is same in both cow (40%) and buffalo (35.94) at all ages. Prevalence of *C. parvum* infection in male and females animals revealed that both the sexes are equally susceptible, which is congruent with Hamnes *et al.* (2007). The prevalence of *C. parvum* infection in confined dairy calves showed a high infection rate than the calves with outdoor access. O' Handley (2007) reported that *C. parvum* is highly prevalent in young dairy calves and confined beef calves, it

Table I. Prevalence of *Cryptosporidium parvum* in cow and buffalo calves in Lahore

Factor	Cow calves (n=250)			Buffalo calves (n=250)		
	No. of calves examined	No. of positive calves	Prevalence	No. of calves examined	No. of positive calves	Prevalence
Overall	250	68	(27.2%)	250	60	(24 %)
Breed **						
Sahiwal	30	6	(20%)	-	-	-
Cross bred	220	62	(28.18%)			
Nili Ravi	-	-	-	250	60	(24%)
Age*						
1-30 days	61	31	(50.81%)	69	29	(42.02%)
1-3 months	65	18	(27.69%)	66	17	(25.75%)
4-8 months	79	14	(17.72%)	60	9	(15%)
9 months-1 year and above	45	5	(11.11%)	55	5	(9.09%)
Sex**						
Male	117	32	(47%)	113	27	(45%)
Female	133	36	(53%)	137	33	(55%)
With/without outdoor access*						
Confined	129	42	(32.55%)	110	35	(31.18%)
Out door access	121	26	(21.48%)	140	25	(17.85%)
Physical condition**						
Healthy	139	43	(30.93%)	108	32	(29.62%)
Weak	83	19	(22.89%)	124	25	(20.16%)
Emaciated	28	6	(21.42%)	18	3	(16.66%)
Feeding *						
Milk Suckling	66	35	(53.03 %)	75	36	(48%)
Fodder eating	90	08	(8.86%)	65	05	(7.69%)
Milk suckling and fodder eating	94	08	(8.86%)	110	19	(17.27%)
Floor condition**						
Paved	108	24	(22.22%)	90	18	(20%)
Dirt yard	142	44	(30.98%)	160	42	(26.25%)
Contact with birds, rodents, dogs, cats*						
With contact	167	52	(31.13%)	217	56	25.80%)
No contact	83	16	(19.27%)	33	4	(12.12%)

* Indicates significant difference (P<0.05) among the groups.
** Indicates non-significant difference (P>0.05) among the groups

Table II. Association of *Cryptosporidium parvum* with diarrhea in dairy calves in Lahore

Category	Age group	Animal			
		Cow calves		Buffalo calves	
		Number examined	No. Positive (%)	Number examined	Positive (%)
	1-30 days	39	23(58.97%)	45	22(48.88%)
Diarrheic*	1-3 months	25	11(44%)	28	10 (35.71%)
	4-8 months	46	10(21.73%)	32	6 (18.75%)
	9 months-1 year& above	30	4 (13.13%)	37	4 (10.81%)
	1-30 days	22	8 (36.36%)	24	7(29.16%)
Non- Diarrheic*	1-3 months	40	7 (17.5%)	38	7 (18.42%)
	4-8 months	33	4 (12.12%)	28	3 (10.71%)
	9 months-1 year& above	15	1(6.66%)	18	1 (5.55%)

* Indicates significant difference (P<0.05) between the groups

occurs rarely in calves on range and in adult cattle. In well-managed herds, clinical disease due to *C. parvum* is also rare. The present study showed a relatively high prevalence (30.28%) in the physically healthy calves, which may be attributed to the fact that the most vulnerable age group of cow calves and buffalo calves (1-30 days old) are predominantly healthy under existing husbandry practices, consequently resulting in a high prevalence in the group than weak (21.5%) and emaciated (19%) calves. A significantly higher prevalence of *C. parvum* infection was observed in calves having some sort of contact with birds/rodents/dogs/cats, which may be due to the fact that these animals may act as carrier of *C. parvum*.

In the present study it was observed that *C. parvum* infection in dairy calves was associated with clinical diarrhea. Thus risk for being diarrheic was significantly higher for animals infected and subsequently shedding *C. parvum* compared to calves not shedding. These observations are in conformity to O'Handley *et al.* (1999), who reported that Cryptosporidium is often the only pathogen detected in diarrheic calves. In the present study, the prevalence of *C. parvum* infection peaked in young calves between 1-30 days in both diarrheic and non-diarrheic groups. Similarly Olson *et al.* (1997) observed

59% of dairy calves up to 24 weeks of age on 20 farms in British Columbia were shedding *C. parvum* oocysts. Singh *et al.* (2006), similarly found 40% of diarrheic and 19% non-diarrheic claves were shedding cryptosporidium oocysts, hence the risk of being diarrheic was 1.59 times greater for animals infected with C. *parvum* as compare to non-infected. Similarly Jose *et al.* (2002) found 47.9% prevalence of cryptosporidium in faeces of calves. Sanford and Josephson (1982) also reported Cryptosporidium infection in 26% neonatal, diarrheic calves, over a 32 months period.

In conclusion, *C. parvum* is prevalent in dairy calves especially in young age groups and it is suggested that practitioners in Lahore should consider *C. parvum* for differential diagnosis when investigating the etiology of diarrhea in young calves (1-30 days).

REFERENCES

Angus, K.W., 1990. Cryptosporidiosis in ruminants. *In*: Dubey, J.P., C.A. Speer and R. Fayer (eds.), *Cryptosporidiosis of Man and Animals*, pp: 83–103. Boca Raton CRC Press

Atwill, E.R., J.A. Harp, T. Jones, P.W. Jardon, S. Checel and M. Zylstra, 1998. Evaluation of periparturient dairy cows and contact surfaces as a reservoir of *Cryptosporidium parvum* for calfhood infection. *American J. Vet. Res.,* 59: 1116–1121

Casemore, D.P., 1991. Laboratory methods for diagnosing cryptosporidiosis. *J. Clin. Pathol.,* 44: 445–451

Current, W.L., 1985. Cryptosporidiosis. *J. American Vet. Med. Assoc.,* 187: 1334–1338

Fayer, R., 1997. *Cryptosporidium and Cryptosporidiosis*, p: 251. CRC Press, Boca Raton

Garber, L.P., M.D. Salman, H.S. Hurd, T. Keefe and J.L. Schlater, 1994. Potential risk factors for Cryptosporidium infection in dairy calves, *J. American Vet. Med. Assoc.,* 205: 86–91

Hamnes, I.S., B.K. Gjerde and L.J. Robertson, 2007. A longitudinal study on the occurrence of Cryptosporidium and Giardia in dogs during their first year of life. *Acta. Vet. Scand*., 49: 22

Henriksen, S.A. and H.V. Krogh, 1985. Bovine Cryptosporidiosis in Denmark. 1. Prevalence, age, distribution and seasonal variation. *Nord. Vet. Med*., 37: 34–41

Jose, A.C.H., A.G.L. Yolanda and A.M. Elvira, 2002. Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Glicia (NW Spain). *Vet. Parasitol.,* 106: 1–10

Larson, L.L., F.G. Owen, J.L. Abright, R.D. Appleman, R.C. Lamb and L.D. Muller, 1977. Guidelines towards more uniformity in measuring and reporting calf experimental data. *J. Dairy Sci.,* 60: 989–991

Leek, R.G. and R. Fayer, 1984. Prevalence of Cryptosporidium infections and their relation to diarrhea in calves on 12 dairy farms in Glicia (NW Spain). *Vet. Parasitol.,* 106: 1–10

Nydam, D.V., E.W. Susan, L.S. Stephanie and O.M. Hussni, 2001. Number of *Cryptosporidium parvum oocysts* or *Giardia* spp cysts shed by dairy calves after natural infection. *J. American Vet. Res*., 62: 1612–1615

O' Handley, R.M., C. Cockwill, T.A. McAllister, M. Jelinski, D.W. Morck and M.E. Olson, 1999. Duration of natural giardiosis and cryptosporidiosis in dairy calves and their association with diarrhea. *J. American Vet. Med. Assoc.,* 214: 391–396

O'Handley, R.M., 2007. *Cryptosporidium parvum* infection in cattle: are current perceptions accurate? *Trends Parasitol*., 23: 477–480

Olson, M.E., N.J. Gussele and R.M.O. O'Handley, 1997. Giardia and Cryptosporidium in dairy calves in British Columbia. *Canadian Vet. J*., 38: 703–706

Reynolds, D.J., J.H. Morgan and N. Chanter, 1986. Microbiology of calf diarrhea in Southern Britain. *Vet. Rec.***,** 119: 34–39

Sanford, S.A. and G.K.A. Josephson, 1982. Bovine cryptosporidiosis: Clinical and pathological findings in forty-two scouring neonatal calves. *Canadian Vet. J.***,** 23: 340–343

Scott, C.A., H.V. Smith, M.M.A. Mtambo and H.A. Gibbs, 1995. An epidemiological study of *Cryptosporidium parvum* in two herds of adult beef cattle. *Vet. Parasitol*., 57: 277–288

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

Sturdee, A.P., A.T. Bodley-Tickell, A. Archer and R.M. Chalmers, 2003. Long-term study of Cryptosporidium prevalence on a lowland farm in United Kingdom. *Vet. Parasitol*., 116: 97–113

Uga, S., J. Matsua, E. Kono, K. Kimura, M. Inoue, S.K. Rai and K. Ono, 2000. Prevalence of *Cryptosporidium parvum* infection and pattern of oocyst shedding in calves in Japan. *Vet. Parasitol*., 94: 27–32

Villacorta, I., J.E. Peeters, E. Vanopdenbosch, E. Ares-Mazas and H. Theys, 1991. Efficacy of halofuginone lactate against *Cryptosporidium parvum* in calves. *Antimicrob. Agents Chemother*., 35: 283–287

Watanabe, Y., C.H. Yang and H.K. Ooi, 2005. Cryptosporidium infection in livestock and first identification of *Cryptosporidium parvum* genotype in cattle faeces in Taiwan. *Parasitol. Res*., 97: 238–241

Young, S., M.C. Healey and C. Du, 1996. Infectivity of preserved *Cryptosporidium parvum* oocysts for immuno compressed adult mice. FEMS *Immunol. Med. Microbiol.,* 13: 141–145

(Received 29 April 2008; Accepted 15 July 2008)