



Prevalence of *Cryptosporidium* in Livestock reared near Mahakali and Karnali River Basins of Western Nepal

Nepal Scholar: Dr. Tapendra Prasad Bohara, Department of Livestock Services

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Abstract

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, goats, pigs and horses (Fayer, 1997). *C. Parvum* has been incriminated as an important cause of diarrhea in neonatal calves (Nydam et al., 2001). It is also an important zoonotic pathogen transmitted primarily through water. A high prevalence of *C. parvum* in the human population has been found in different parts of Nepal (Sherchand et al, 1995), however very little information is available on the prevalence of *Cryptosporidiosis* in western Nepal. Moreover, Nepal is prone to climate change impacts and climatic parameters are closely associated with the maturation and infectivity of enteric protozoa such as *cryptosporidium* and *cyclospora*. This study endeavored to bridge the knowledge gap in this area. Farmers lose a number of younger calves primarily due to the problem of diarrhea, *cryptosporidiosis* may be a factor. Effective drugs for control and treatment of *cryptosporidiosis* in livestock and poultry are not available, but effective sanitary practices for prevention can be utilized if prevalence is confirmed. This will help farmers to minimize the economic losses due to calf mortality as well as the zoonotic potential of the parasite. 🐄

Sampling in the Mahakali and Karnali River Basins



Typical buffalo farm in the study area. (Photo Credit: Tapendra Prasad Bohara)

A cross sectional study was conducted from November, 2014 to June, 2015 in calves of cattle and buffalo reared near the river basins of Mahakali and Karnali. Samples were examined using the Ziehl-Neelsen Staining technique. Of 350 fecal samples tested, 170 were positive showing an overall prevalence of 48.6% for *Cryptosporidium* in cattle and buffalo calves. Cow calves (50.3%) had a higher prevalence than the buffalo calves (47.3%). The prevalence of infection was highest in the 3-6 months (53.7%) age group for both cow and buffalo calves. Males had a higher prevalence of infection (53.1%) than females (45.3%). Exotic animals had a higher prevalence of infection (50%) than did the indigenous breeds. The highest prevalence was observed in Bardiya (57.7%) followed by Kanchanpur and Kailalil; the Karnali river basin had a higher incidence than the Mahakali river basin. Thatched sheds, more grazing hours and use of surface water also increased the prevalence of *Cryptosporidium*. A standard questionnaire was designed for the exploration of risk factors associated in calves and human beings for *cryptosporidium* infection. 350 questionnaires were administered randomly in populations living near the river basins and the national parks and wildlife reserves in the Bardiya, Kailali and Kanchanpur districts. The households used for questionnaires were included in the fecal sample collection also. A total of 350 fecal samples of calves of cattle and buffalo living in the districts were collected by random methods. The fecal samples were collected directly from the rectum of each calf, or a fresh sample was taken from the ground where available and if calves were housed individually. Samples were enclosed in self-sealing polythen bags and preserved in ice until refrigerated at 4C in the laboratory. Each sample was run through an oocyst floatation technique using sucrose solution with a specific gravity of 1.18 (Uga et al., 2000).



Table 1. Prevalence of Cryptosporidiosis in relation to animal factors in cattle and buffalo calves

Factor	Cattle calves (n=143)			Buffalo calves (n=207)			Total calves (n=350)		
	No. of calves examined	No. of positive cases	Prevalence	No. of calves examined	No. of positive cases	Prevalence	No. of calves examined	No. of positive cases	Prevalence
Age									
0-3 Months	47	24	51.1	71	29	40.8	118	53	44.9
3-6 Months	83	43	51.8	105	58	55.2	188	101	53.7
6-9 Months	7	3	42.9	12	5	41.7	19	8	42.1
9-12 Months	6	2	33.3	19	6	31.6	25	8	32.0
Sex									
Male	63	37	58.7	84	41	48.8	147	78	53.1
Female	80	35	43.8	123	57	46.3	203	92	45.3
Breed									
Indigenous	132	65	49.2	56	23	41.1	188	88	46.8
Exotic	11	7	63.6	151	75	49.7	162	82	50.6
OVERALL	143	72	50.3	207	98	47.3	350	170	48.6

Two grams of fecal material were weighed using an electric balance. Fecal material was added in 3 ml of fecal floatation fluid in a 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 minutes. A small drop from the top was transferred to a clean, labeled glass slide to make a smear. The smears were stained by modified Ziehl-Neelsen (mZN) acid fast staining as described by Casemore (1991) at the Animal Health Research Division of the Nepal Agricultural Research Council (NARC), Khumaltar and Central Veterinary Laboratory, Tripureshwor. Chi-square tests were used to determine significant association of risk factors with the prevalence of Cryptosporidium in cattle and buffalo calves. Probability (P) value of <0.05 was defined as statistically significant.

Results and discussion

Prevalence of cryptosporidium infection in calves is shown in Table 1 and 2 in relation to animal factors and management practices respectively, social and geographical factors were also analyzed and are shown in Table 3. Out of 350 fecal samples tested, 170 were positive for overall prevalence at 48.6%. Bhat et al., (2012) reported a prevalence of 38.3% in neonatal calves of Punjab, India. Sturdee et al., (2003) reported a 52.4% prevalence in home-bred calves on a low land farm in the UK. This finding is slightly higher than the findings of Paudyal et al., (2013) in calves of the Kathmandu Valley and Maurya et al., (2013), in India. A relatively high prevalence in calves may be due to a difference in climatic and management conditions. Cow calves had a higher prevalence than did buffalo calves. This finding is congruent with the findings of Nasir et al., 2009. Singh et al., 2006 similarly found that pathogenicity of *C. parvum* is at 40% in cow calves and 35.94% in buffalo calves. The prevalence was higher in exotic cattle calves than indigenous cattle calves.

Table 2. Prevalence of Cryptosporidiosis in relation to social and geographical factors

Factor	Cattle calves (n=143)			Buffalo calves (n=207)			Total calves (n=350)		
	No. of calves examined	No. of positive cases	Prevalence	No. of calves examined	No. of positive cases	Prevalence	No. of calves examined	No. of positive cases	Prevalence
Ethnicity of owner									
Tharu	34	21	61.8	19	6	31.6	53	27	50.9
Brahmin	27	13	48.1	68	38	55.9	95	51	53.7
Chhetri	64	31	48.4	105	51	48.6	169	82	48.5
Dalits	18	7	38.9	15	3	20.0	33	10	30.3
Districts									
Bardiya	56	33	58.9	74	42	56.8	130	75	57.7
Kailali	36	15	41.7	46	16	34.8	82	31	37.8
Kanchanpur	51	24	47.1	87	40	46.0	138	64	46.4
River basin									
Karnali	92	48	52.2	120	58	48.3	212	106	50.0
Mahakali	51	24	47.1	87	40	46.0	138	64	46.4
OVERALL	143	72	50.3	207	98	47.3	350	170	48.6



Giving the questionnaire survey. (Photo credit: Tapendra Prasad Bohara)

Considering age as a factor, the prevalence of infection was highest in the 3-6 months age group in both cattle and buffalo calves. The 0-3 months age group had the next highest infection rate, followed by 6-9 months and then the 9-12 months age group. The prevalence of infection was significantly higher ($p < 0.05$) in the 1-30 days age group of both the cow and buffalo calves followed by 1-3 months, 4-8 months and 9 months to 1 year and above (Nasir et al. 2009). These results are in line with Santin et al (2004) and Fayer et al. (2007) who suggested that the mature dairy cattle are at low risks of infections as compared to preweaned calves.

A higher prevalence of *Cryptosporidium* infection was observed in male calves than female cow and buffalo calves, 53.1% and 45.3%

respectively. Paudyal et al. (2013) observed a much larger difference in rate of infection between the sexes; 11.8% of female calves and 42.4 % of male calves were positive in the Kathmandu Valley. This reveals that males have a higher chance of acquiring the parasitic infection. Prevalence of infection was observed in relation to various management practices followed by the farm. The prevalence was higher in the farms with thatched sheds (49.1%), more hours of grazing (52.2%), grazing on river size (54.5%) private lands (56.7%) and use of surface water sources (52.6%) for livestock. As all of these factors are closely related to sanitation, such factors might contribute to the transmission of parasites.

Conclusions

In summary, this research project has shown that *Cryptosporidiosis* is evident in the cattle and buffalo populations of western Nepal. *Cryptosporidium* in wild and domestic animals has also been reported in Nepal (Karna, 2010). A high prevalence of *C. parvum* in human populations has been found in different parts of Nepal, including Jomsom, Kathmandu Valley and Chitwan (Sherchand et al., 1995). Infection caused by *C. Parvum* occurred in people of all ages, but most cases were reported in children less than 5 years of age (Sherchand and Shrestha, 1996). As the parasite has the potential for zoonotic transmission, *Cryptosporidiosis* must be ruled out while diagnosing the enteric infections in livestock and human beings. Moreover, better sanitary practices should be utilized in human settlements and the livestock farms of the area. Further studies are needed to identify the species of the parasites with molecular techniques and exploration of various risk factors associated with the transmission of parasites in both the livestock and human populations. 🐄

Table 3. Prevalence of Cryptosporidiosis in relation to management practices

Factor	Cattle calves (n=143)			Buffalo calves (n=207)			Total calves (n=350)		
	No. of calves examined	No. of positive cases	Prevalence	No. of calves examined	No. of positive cases	Prevalence	No. of calves examined	No. of positive cases	Prevalence
Shed type									
Thatched	126	64	50.8	165	79	47.9	291	143	49.1
Concrete	16	7	43.8	42	19	45.2	58	26	44.8
Drinking Water									
Surface water	83	44	53.0	111	58	52.3	194	102	52.6
Underground water	60	28	46.7	96	40	41.7	156	68	43.6
Grazing land									
Community Forest	41	19	46.3	60	23	38.3	101	42	41.6
Conservation park/reserve	32	14	43.8	49	21	42.9	81	35	43.2
River side	56	33	58.9	45	22	48.9	101	55	54.5
Private land	14	6	42.9	53	32	60.4	67	38	56.7
Husbandry practice									
Grazing hrs=Stall feeding	17	6	35.3	32	14	43.8	49	20	40.8
Grazing hrs>Stall feeding	98	55	56.1	132	65	49.2	230	120	52.2
Grazing hrs<Stall feeding	28	11	39.3	43	19	44.2	71	30	42.3
OVERALL	143	72	50.3	207	98	47.3	350	170	48.6

Further Reading

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TIRI, Targeted Investment for Research Impact, identifies early-career researchers who are interested in tackling livestock production problems through innovative approaches and fresh perspectives. This small-grant program is open to early-career researchers (five or fewer years into research career) in any discipline, from student to professor, and from any organization that is engaged in applied research on livestock production in South Asia and East Africa — colleges and universities, government research centers or laboratories, or non-profit organizations.

Proposals are selected based on their potential to make livestock production systems more resilient to increasing climate variability and severity. At the end of one year, TIRI scholars are expected to demonstrate concrete outcomes and real potential for future impact. The 10 selected East Africa TIRI scholars and the 18 selected Nepal TIRI scholars are addressing research problems on various livestock and climate research themes.



Feed the Future Innovation Lab for Collaborative Research on Adapting Livestock Systems to Climate Change is dedicated to catalyzing and coordinating research that improves the livelihoods of livestock producers affected by climate change by reducing vulnerability and increasing adaptive capacity.

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