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ORIGINAL ARTICLE

Prevalence and risk factors associated with *Giardia duodenalis* infection in dairy cattle of Chitwan, Nepal

M. K. Mahato[1] · D. K. Singh[2] · H. B. Rana[2] · K. P. Acharya[2,3]

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Abstract Livestock farming has been an integral part of Nepalese agriculture systems since time immemorial. *Giardia duodenalis* is a cosmopolitan intestinal parasite present in a wide range of hosts. Very little or no information is reported on the prevalence of giardiasis in livestock of Nepal. This study was done during Jan 18 to July 19, 2014 to determine the prevalence and associated risk factors of *Giardia duodenalis* infection in dairy cattle of Chitwan, Nepal. A total of 96 fresh fecal samples were collected from various dairy pocket areas and were transferred to collection bottles with 10% formalin. Wet smears of the samples were prepared, stained with lugol's iodine and then viewed under microscope at 400× magnification. The overall prevalence was found to be 44.79% (43/96). Based on the risk factors assessment survey, age was found to be significantly associated with the prevalence of *Giardia duodenalis*. The prevalence was found significantly ($P < 0.05$) higher in 1–6 months age group compared to > 3 years. Similarly, higher prevalence was found in diarrheic animals compared to their counterparts ($P < 0.05$). Though higher prevalence was recorded in unhygienically housed animals, it was statistically non-significant ($P < 0.05$). Giardiasis should be considered as an important cause of diarrhea and further advanced diagnostic approaches should be employed for the confirmation of giardiasis in dairy cattle.

Keywords *Giardia duodenalis* · Risk factors · Cattle · Nepal

Introduction

Since long, giardiasis has been a well known gastrointestinal disease in both humans and animals characterized by acute or chronic diarrhea. The causative agent is a protozoan parasite of the genus *Giardia*. *Giardia duodenalis* is the most commonly encountered parasite associated with gastrointestinal disease in the ruminants (Cacciò et al. 2005; Smith et al. 2007). Giardiasis in the ruminants may be the causative factor for reduced growth, reduced performance and for production losses. The decreased growth, performance and production loss is due to acute or chronic diarrhea but some animals either may remain asymptomatic (Waller 1999) or not associated with diarrhea (St. Jean et al. 1987; O'Handley et al. 1999; Huetink et al. 2001; Bjorkman et al. 2003). In case of cattle, sheep and goats infection may prolong to chronic cases (Koudela and Vitovec 1998; Sweeny et al. 2011). Giardiasis has been reported from across the world with high prevalence in calves (10–89%) but comparatively persistent and lower prevalence was recorded in adults (Becher et al. 2004; Trout et al. 2005; O'Handley et al. 2000; Olson et al. 2004). High level of variations in its prevalence has been found due to the differences in the study design, sampling sizes and

M. K. Mahato and K. P. Acharya have contributed equally to this work.

✉ K. P. Acharya
kriaasedu@gmail.com

[1] Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal

[2] Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan, Nepal

[3] Regional Veterinary Diseases Diagnostic Laboratory (RVDDL), Pokhara, Nepal

strategies (Sweeny et al. 2011) besides variations in the management and climatic conditions (Xiao 1994).

Nepal is an agricultural country where more than 65% of the people are engaged in farming. Livestock farming is an integral part of the Nepalese farming system and it contributes 11.5% of the national GDP. Diarrhea and associated disorders have been the most common issues among cattle population in Nepal. Oftentimes these cases are diagnosed as helminthiasis. Lack of researches in this aspect in Nepal could be one of the major causes of this uncertainty and negligence. Thus, this study was done to report the prevalence of *Giardia duodenalis* and its associated risk factors in cattle population of Chitwan district of Nepal.

Materials and methods

Study design

A cross sectional study was performed in the site stated for a period of six months (Jan 18 to July 19, 2014). A survey containing both open and closed ended questionnaire was used to assess the associated risk factors. Farm facilities, design and condition were collected through direct observation. Personal health information was collected to survey zoonotic potentiality of the parasite. The study was completed in a period of 6 months.

Sampling procedure and size

Age stratified random sampling technique was used to take samples from different areas of Chitwan, Nepal. Four strata of age groups were created: 1–6 months of age calves), 7–12 months of age young stock), 1–2 and > 3 years (Wade et al. 2000). Sample size was calculated by assuming the prevalence to be 50%, at desired precision to be 10. At 95% confidence level, the sample size is given by the formula,

$n = 1.96^2P(1 - P)/d^2$ (Daniel 1999)

p = prevalence and d = desired precision

I.e. n = 96 is the sample size

Diagnosis

Formalin-ethyl-acetate sedimentation and Zinc sulphate floatation techniques with lugol's iodine staining or without staining were employed for the fecal examination as per Soulsby. The wet smears were viewed under 100× and 400× magnifications.

Data analysis

All the data were entered using a computer software MS excel 2007. The qualitative survey data was converted into quantitative for statistical analysis. Association of independent variables species, sex, and age) and prevalence of *G. duodenalis* was evaluated using statistical software Open Epi version 2.3. All statistical analyses were performed at 95% level of confidence and *P* value less than 0.05 was considered as significant. Chi square test and Odds ratio were employed to test and measure the degree of association between different variables, respectively.

Results and discussion

Giardia duodenalis appeared as oblique cyst having ventral concavity and two prominent nuclei at anterior portion of cyst (Fig. 1). The overall prevalence was found to be 44.79% (43/96). The result of this study is in accordance to the findings of multicentre studies of prevalence of *Giardia duodenalis* done in four major cattle rearing European countries–Germany, UK, France and Italy (Geurden et al. 2012), Ontario Canada (Coklin et al. 2007 and Ruwanda (Hogan et al. 2014). However it is higher than the studies done in some countries like New Zealand North Island (Hunt et al. 2000), New Zealand South Island (Winkworth et al. 2008), USA-East coast (Trout et al. 2006), Pakistan

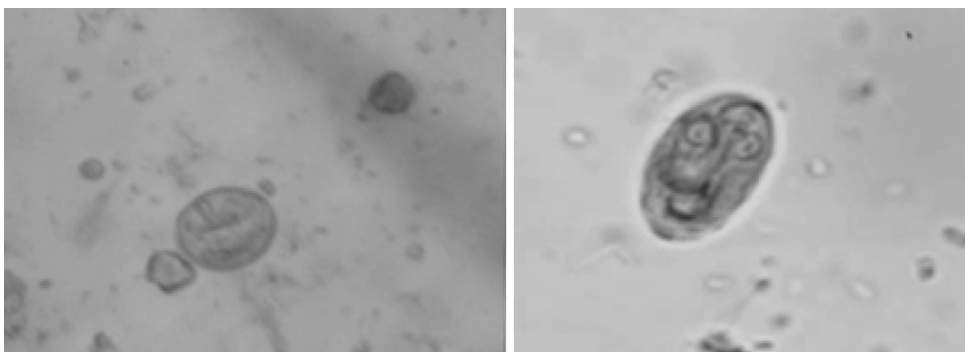

Fig. 1 Giardia cysts under ×400 magnifications in unstained fecal wet smears. It is egg shaped measuring approx. 8–14 μm by 7–10 μm; each cyst contains four nuclei, four median bodies and 8 pairs of flagella

Table 1 Prevalence associated with animal risk factors

Risk factors		No. of animals tested	No. of animals positive	Prevalence (%)	OR	95% CI	*P* value
Age	1–6 months	29	19	65.52		47.35–80.06	0.01983*
	7–12 months	21	10	47.62		28.34–67.63	
	1–3 years	21	8	38.10		20.75–59.12	
	> 3 years	25	6	24.00		11.50–43.43	
Sex	Male	21	10	47.62	1.16	28.34–67.63	0.7682
	Female	75	33	44.00		33.33–55.25	
Health	Diarrheic	16	11	68.75	3.30	44.40–85.84	0.03477*
	Non-diarrheic	80	32	40.00		29.96–50.95	

OR odd ratio, *CI* confidence interval

*$P < 0.05$

(Ahmad et al. 2015), Portugal (Mendonça et al. 2007) but is comparatively lower than the findings another study of American East coast (Coklin et al. 2007) and Australia (Becher et al. 2004). These differences in prevalence may be due to the differences in type of farming practices followed e.g. in Nepal traditional and unhygienic farm practices is prevalent. On the other hand, higher temperature and precipitation level in spring and summer season, and presence of swampy areas in the site of study may explain reason of higher prevalence rate to some extent. It is well known that increased precipitation will expand the areas contaminated by giardia cysts and may create favorable environment for the transmission of giardia cyts (Alum et al. 2014; Zhang et al. 2016). Hence, higher precipitation and higher temperature may have contributed to high prevalence rate in our study. Besides, sampling size the differences in diagnostic methods may have direct effects on prevalence rate.

The prevalence rate as a result of this study was significantly ($P < 0.05$) higher in the age group of 1–6 months compared to the age group greater than 3 years (Table 1) which is in agreement with the findings from Pakistan (Maqbool et al. 2008), Canada (Gow and Waldner 2006), Europe Germany, UK, France and Italy) (Geurden et al. 2012), New York (Mark-Carew et al. 2010) and Spain (Castro-Hermida et al. 2006 and China (Liu et al. 2012). The prevalence of *G. duodenalis* was found in decreasing trend with the increase in age of the animal which in agreement with the findings of multicentre studies in the Europe (Geurden et al. 2012) and the Sicily island (Piazza et al. 2013) but in contrast with reports from Denmark (Maddox-Hyttel et al. 2006) and New Zealand (Uehlinger et al. 2005). These differences in the results may be associated with the health condition and history of use of anthelminthics and antiprotozoals. Higher infections in young animals may be related to lack of protective immunity. Prevalence of *Giardia duodenalis* was found higher in males than females. This finding is supported by the results of Bangladesh (Suman et al. 2008). But this finding is contrasting with the report from Lahore, Pakistan (Maqbool et al. 2008). The reason behind this difference may be associated with the health status of animals during sampling. There was a significant difference ($P < 0.05$) in the prevalence of *G. duodenalis* in diarrheic and apparently healthy animals. Similar result based on fecal consistency was obtained in Italian calves (Piazza et al. 2013). Higher prevalence rates were associated with unhygienic farm conditions like lesser shed-manure pit distance, lesser shed-water source distance, muddy shed floor and lesser shed cleaning frequency; however, it was statistically non-significant (Table 2). Farm hygiene parameters could also play an important role in the prevalence of this protozoan parasite.

Conclusion

The overall prevalence of *G. duodenalis* is high in Nepal. As shown by most of the studies, young calves are more susceptible to this disease. The reason behind this may be due to the immature immunity of the young calves. Though the farm hygiene parameters do not show significant relation statistically but the study shows higher prevalence in unhygienic conditions. These farm hygiene parameters need to be strictly improved in farm practices of Nepal. The prevalence of *G. duodenalis* is statistically significant in the case of diarrheic animals. This is the major point to be considered that giardiasis should not be discarded as differentials for the cause of diarrhea and further diagnostic approaches should be employed for confirmation. *Giardia duodenalis* could be the sole cause of diarrhea or may be associated with the multi-factorial cause of gastrointestinal disorders. Young stocks should be given special care to

Table 2 Prevalence associated with farm risk factors

Risk factors		No. of animals tested	No. of animals positive	Prevalence (%)	Odds ratio	95% CI	*P* value
Floor type	Cemented	84	36	42.86		32.82–53.52	0.4293
	Bricked	8	4	50.00		21.52–78.48	
	Muddy	4	3	75.00		30.06–95.44	
Shed-manure pit distance	Attached	13	9	69.23		42.37–87.32	0.1578
	< 5 m	27	12	44.44		27.59–62.69	
	> 5 m	56	22	39.26		27.58–52.37	
Water-manure pit distance	< 10 m	32	15	46.88	1.13	30.87–63.55	0.7716
	> 10 m	64	28	43.75		32.29–55.91	
Shed cleaning frequency	< 3 times	72	33	45.83	1.19	34.83–57.26	0.722
	> 3 times	24	10	41.67		24.47–61.17	

OR odd ratio, *CI* confidence interval

*$P < 0.05$

prevent exposure from any risk factors as they are highly susceptible. Diarrhea in young stocks may be associated with giardia hence fecal examination for Giardia is recommended.

Compliance with ethical standards

Conflict of interest Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

Ethical approval All the samples were collected with the due consent of farm owners and no animals were harmed throughout the research period. All the research activities done in animals were performed as per the ethical guidelines for care and use of animals in health research in Nepal set by Nepal health research council.

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