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Full Length Research Paper

Prevalence of small ruminant gastrointestinal parasites infections and associated risk factors in selected districts of Bale zone, south eastern Ethiopia

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Parasitic infections pose a serious health threat and remain one of the major impediments to small ruminant production in many part of the world including Ethiopia. Given the huge economic burden of the disease, a comprehensive study covering a wider study area is of paramount importance to generate accurate information about the disease. The current study was therefore, designed with the objectives to determine the prevalence, species involved and assesses the associated risk factors of gastrointestinal parasites (GIT) of Small ruminants in Bale zone. A cross-sectional study was conducted from January to May 2016 in the purposively selected three districts of Bale zone, Southern Eastern Ethiopia. Faecal samples were randomly collected from 384 shoats (41 sheep and 343 goats) and examined coprologically. Logistic regression was used to determine the association of risk factors with positivity for GIT parasite. The study revealed an overall prevalence of 77.8% of which 63.4 and 79.6% were sheep and goats, respectively. Nine genera of parasites with the overall prevalence of Strongyloides (25.2%), Trichostrongylus (13.8%), Coccidia (15.1%), Paramphistomum (14%), Fasciola (11.5%), Ostertagia (1.5%), Haemonchus (1%), Trichuris (0.26%) and Oesophagstamum (0.26%) with mixed infection (17%) were identified in the area. By categorizing parasites, Nematode, Trematodes and Eimeria were found to infect the small ruminants in the area with the overall prevalence of 40.8, 23 and 14% respectively. Logistic regression analysis showed that the risk of GIT parasite was significantly higher in goats than sheep (OR: 2.821, CI=1.27- 6.23, *P*=0.010) and in adult than young shoats (OR=2.19, CI= 1.296-3.714, *P*=0.003). The body condition was also significantly associated with risk of positivity for GIT parasite (*P*=0.004). However, there was no significant difference in prevalence between sex and district of the study animals. Overall prevalence in districts level was found to be 81.9, 74.6 and 76.9% in Madda Walabu, Haranna Buluk and Dallo Manna districts, respectively. From the studied animals, 37.8, 29.8 and 32.2% were lightly, moderately and heavily infested, respectively. This study thus revealed that polyparasitism is a major health problem and hindrance in small ruminants' production in current study area. Therefore, periodic and strategic deworming intervention with effective broad spectrum anti-helminths, awareness creation, proper grazing system and stocking size encompassing all localities of the study area is needed to mitigate this daunting problem.

Key words: Small ruminants, goat, sheep, gastrointestinal parasites, Bale, Ethiopia

INTRODUCTION

Ethiopia possesses an estimate of 28.89 million sheep and 29.7 million goats (CSA, 2016) which are well adapted to local climatic and nutritional conditions and contribute greatly to the national economy (Alemayehun and Fletcher, 1995). Sheep and goat are integral to the livestock production systems in crop-livestock mixed agriculture in the highlands and in the pastoral and agro-pastoral livestock production. They are particularly important resources of the country as they provide more than 30% of the local meat consumption and form a vital source of income for small-scale farmers (ILCA, 2007).

However, the benefits obtained from sheep and goats to date do not match their tremendous potential and significant losses resulted each year from the death of animals as a result of lack of appropriate veterinary services, lack of attention from government and wide spread endemic diseases which are considered as bottleneck for development of this sector in the country (Dabassa et al., 2013; Abdela and Jilo, 2016; Jilo et al., 2016; Abdela, 2016, 2017). By and large, parasitic infections pose a serious health threat and limit the productivity of livestock due to the associated morbidity and mortality (Abede and Esayas, 2001; Cernanska et al., 2005). More specifically, plethora of parasitic diseases plays a detrimental role in hampering small ruminant production leading to serious economic loss (Cernanska et al., 2005).

Worldwide, parasite helminthes are major cause of losses in productivity and health problem of goat and sheep and usually associated with huge economic losses especially in resource poor region of the world (Cernanska et al., 2005). Parasite helminthes also cause susceptibility to other diseases (Kumba et al., 2003; Githigia et al., 2005). The effect of infestation by gastrointestinal helminthes varies according to the parasite concerned, the degree of infestation and other risk factors such as species, age, season and intensity of worm burden (ILCA, 1990). The problem is much more severe in tropical countries due to very favorable environmental condition for parasite transmission, poor nutrition of host animals, and poor sanitation in facilities where animals are housed. As a result diseases caused by helminthes remain one of the major impediments to small ruminant production in tropics (Kumsa and Abebe 2009). In the tropics, up 95% of sheep and goat are reported to be infected with helminthes of which Haemonchus and Trichostrongylus are the two most common involved genera (Opara et al., 2005; Gathuma et al., 2007; Mbuh et al., 2008).

Helminthosis of sheep and goat is among the endoparasite infections that are responsible for economic losses through reduced productivity and increased mortality (Perry et al., 2002). The loss through reduced productivity is related to reduction of food intake, stunted growth, reduced work capacity, cost of treatment and control of helminthosis (Pedreira et al., 2006) and losses from clinical and sub-clinical level including losses due to inferior weight gains, lower milk yields, condemnation of organs and carcasses at slaughter and mortality in massively parasitized due to parasitic diseases were documented (Regassa et al., 2006).

Although considerable work has been done on gastrointestinal parasites of sheep and goats in many parts of Ethiopia (Regassa et al., 2006; Kumsa and Abebe, 2009; Zeryehun et al., 2012; Zeleke et al., 2013; Gizachew et al., 2014; Nana, 2016), most of the studies were restricted to only small study areas with limited GIT parasites (Bitew et al., 2010; Zeleke et al., 2013; Gizachew et al., 2014; Melkamu and Asrat, 2015; Nana, 2016). Thus, there is scarcity of study on gastrointestinal parasites of small ruminants in Ethiopia in general and no report so far has been published from the current study areas. However, given the huge economic burden of the disease, a comprehensive study covering a wider study area is of paramount importance to generate accurate information about the disease and thereby, design effective disease control and prevention strategies accordingly. Therefore, the objectives of this study were to determine the prevalence of GIT parasite of small ruminants, identify major GIT parasites and to assess the possible risk factors encompassing wider areas with a wide spectrum of GIT parasite in selected districts of Bale zone.

MATERIALS AND METHODS

Study area

The study was conducted from March to May 30, 2016 in three districts of Bale zone, namely Madda Walabu, Dallo Manna and Haranna Buluk. Bale zone is located in Oromia regional state at a distance about 650 km to South East of Addis Ababa. Resource use in the Bale is largely communal though with crop cultivation and private enclosures that appear to be increasing in recent decades. The area receives bimodal pattern of rainfall, characterized by wide variety of geomorphic landscape and has eight major agro-ecological zones and eleven sub-ecologies spatial and temporal variability in both the quantity and distribution of rainfall renders the area semi-arid, with an average annual rainfall ranging from 600 to 2300 mm in the north (Figure 1).

The average temperature varies from 0 to 30°C per annum. Bale zone has about 1,566,521 cattle, 298,293 sheep, 726,394 goats, 90,685 horses, 17,272 mules, 239,705 donkeys, 710,593 poultry and 190,847 beehives (CSA, 2016).

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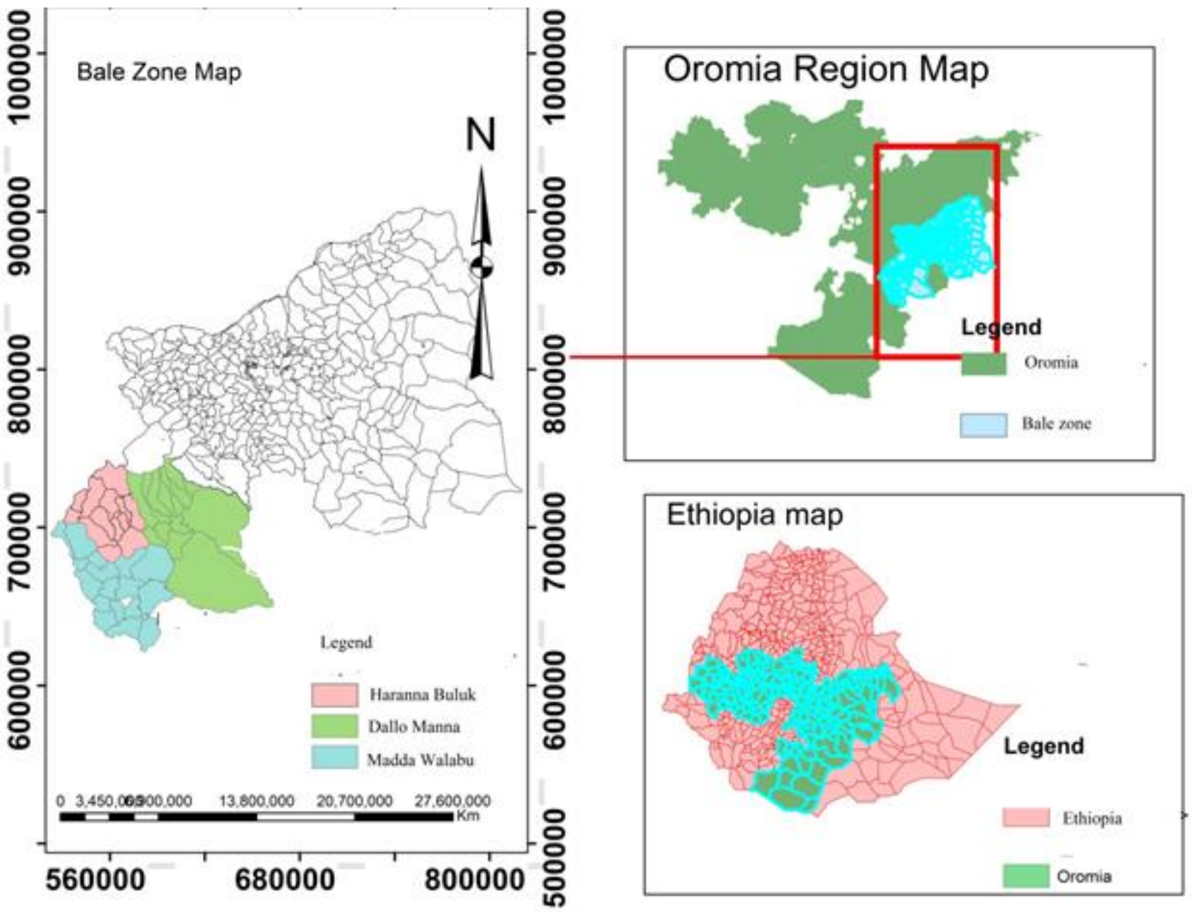

Figure 1: Map of Bale zone showing study districts

Study design and study population

Cross-sectional study design was used to determine the prevalence of GIT parasite of sheep and goat in the three districts of Bale zone. The study animals were sheep and goat in eight Peasant associations of three districts namely, Madda Walabu (Ella Bidire, Madda and Karjul); Dallo Manna (Chirri, Malka and Amana) and Haranna Buluk (Sodu Waymal, Kumbi and Shawe). All of the study animals were kept under traditional production system, featured by extensive pastoral management system where there is also agro pastoralism. Animals biodata like species, sex, age and body condition of the both species were recorded in a format prepared this purpose. Age of study animal was determined based on dentition by eruption pattern as per described by Steel (1996). Conventionally, the age was grouped as young (<2 years) and adult (≥2 years). Body condition of the animals was classified as poor, medium and good based on the appearance of animal and manual palpation of the spinus and transverse processes of lumbar vertebrae as described by Thomson and Meyer (1994).

Sample size determination and sampling technique

The desired sample size for the study was calculated using the formula given by Thrusfield (2005) with 95% confidential interval and 5 % absolute precision.

$$N = 1.96^2 * P_{exp} (1\text{-}Pexp) / D^2$$

Where; n =sample size, P_{exp}=expect prevalence, D=absolute precision (5%).

There was no previously published and documented prevalence in the study area. Therefore, sample size was calculated using expected prevalence of 50% by substituting the value, the required sample size was calculated to be 384 and it was collected accordingly. Simple random sampling technique was employed for selection of animal and study areas in the zone. About 10 g of fecal sample was randomly collected directly from the rectum of sheep and goats those were not dewormed for three months. The collected fecal sample were placed in universal bottles and preserved by using potassium dichromate and labeled appropriately then dispatched to Yabello Regional Veterinary Laboratory within 24 h for carpological examination. Qualitative (flotation and sedimentation) and quantitative examinations (MCmaster egg counting techniques) were employed according to Hansen and Perry (1994). The degree of infection was categorized as light moderate and severe (massive) according to their egg per gram of faeces (EPG) counts. Egg counts from 50-799, 800 1200 and over 1200 eggs per gram of feces were considered as light, moderate and massive infection, respectively (Hansen and Perry, 1994).

Statistical analysis of data

All collected data were entered to Micro-Soft Excel sheet 2010 and analyzed by SPSS version 20. Descriptive statistics was used to

Table 1. Logistic regression analysis output of risk factors associated with GIT parasite of sheep and goats in selected districts of Bale zone.

Risk factors		No. examined	No. positive	Prevalence (%)	Univariable		Multivariable	
					COR (95% CI)	P-value	AOR (95% CI)	P-value
Species	Goat	343	273	79.6	2.25 (1.131 - 4.475)	0.021	2.82 (1.27 - 6.23)	0.010
	Sheep	41	26	63.4				
Age	Adult	231	189	81.8	1.759 (1.082 - 2.859)	0.023	2.19 (1.296 - 3.714)	0.003
	Young	153	110	71.9				
Sex	Male	87	69	79.3	1.117 (0.622 - 2.006)	0.712	1.409 (0.745 - 2.663)	0.291
	Female	297	230	77.4				
Body condition	Good	125	109	87.2	Reference		Reference	
	Medium	225	166	65.0	0.352 (0.142 - 0.871)	0.010	0.278 (0.107 - 0.724)	0.004
	Poor	34	24	70.5	0.413 (0.226 - 0.755)		0.37 (0.201 - 0.707)	
District	MaddaWalabu	133	109	81.9	1.54 (0.857- 2.782)		1.874 (1.008 - 3.484)	
	Harana Buluk	117	90	76.9	1.13 (0.635 - 2.024)	0.341	1.316 (0.673 - 2.572)	0.139
	Dallo Manna	134	100	74.6	Reference		Reference	

COR =crude odd ratio, AOR= adjusted odd ratio, CI =Confidence Interval.

determine the frequency and percentage of both dependent and independent variables. The prevalence was calculated as percent of infected animals from the total number of animals examined. Logistic regression was applied to assess association of risk factors and strength of association. For statistical analysis, a confidence level of 95% and a *P*-values less than 5% were judged as significant.

RESULTS

The overall prevalence of gastrointestinal parasites in small ruminants in this study was 77.8% (299/384) while prevalence in goats and sheep were 79.6% (273/243) and 63.4% (26/41), respectively. By category, Nematode, Trematodes and Eimeria parasites were found to infect the small ruminants in the area with the overall prevalence of 40.8, 23 and 14% respectively (Table 5). The study revealed the statistically significant variation of prevalence of GIT parasite between species in which goats were 2.821 time more likely to be positive for GIT parasite than sheep (*OR*: 2.821; CI=1.27- 6.23, *P*=0.010). Body condition scores (p=0.004) and age (p=0.003) of study animals were also significantly associated with positivity for GIT parasite. Likewise, adult animals were 2.19 more likely to be prone to GIT parasite than young animals (OR=2.19, CI= 1.296-3.714, P=0.003) (Table 1).

The present study revealed statistically significant variation of prevalence of GIT parasites in relation to the body condition of animals as animal with good body condition was found more likely harbors GI parasite than poor and medium body conditioned animals (Table 4). In overall degree of infestation, 32.2% of animals were heavily, 29.8% moderately and 37.8% of animals were lightly infested (Table 4) and up on categorized parasites in to genera; nematode parasites were found the most heavily infesting parasite followed by highest degree of moderately and lightly infesting *Eimeria* species (Table 7).

Regarding GIT parasite prevalence distribution among districts the highest prevalence was recorded in Madda Walabu, 81.9% (109/133) followed by Haranna Buluk, 76.9% (90/117) and Dallo Manna district, 74.6% (100/134) which were dominated by Nematode parasites followed by Trematodes and *Eimeria* species respectively in each districts (Tables 2 and 5). However, this variation was not statically significant (p=0.139).

Table 2. Prevalence of GIT parasite at districts and peasant association level.

District	Peasant associations	Prevalence	Overall prevalence
MaddaWalabu	Ella Bidire	97.7%(44/45)	81.9 %
	Madda	68%(32/47)	
	Karjul	80.4%(33/41)	
Harana Buluk	SoduWaymal	77.0%(37/48)	76.9%
	Kumbi	57.1%(4/7)	
	Shawe	79.0%(49/62)	
Dallo Manna	Chirri	81.4%(57/70)	74.6%
	Malka Amana	67.1%(43/64)	

Concerning the prevalence at peasant associations (smallest administrative unit in Ethiopia) level heterogenic prevalence was recorded ranging from 57.1 to 97.7% in different peasant associations of the study districts (Table 2). Concerning species of parasites in study area Strongloides (25%), Trichostrongylus (13.8%), Coccidia (15.1%), Paramphistomum (14%), Fasciola (11.5%), Ostertagia(1.65%), Haemonchus (1%),Trichuris (0.7%) and Oesophagastamum (0.26%) with mixed infection (17.4%) were the most prevalent GIT parasites (Table 3).

In relation to the species of animal to parasites identified all three genera: Nematode, Trematodes and Eimeria were more prevalent in goats than sheep (Table 6). Regarding the relationship of age with specific genera of parasite; Nematodes were higher in adult; whereas Trematodes and Eimeria were higher in young animals (Table 6). In sex wise prevalence, Nematodes and Trematodes were found higher in female while Eimera oocysts were higher in male animals. This study revealed that males were 1.409 times more likely to be positive for GIT parasite than females. However, this variation were not statically significant during multivariable logistic regression analysis (OR=1.409, CI=0.745- 2.663, *P*= 0.291) (Table 6).

At the specific species, Strongloides (25.36 and 24.4%), Trichostrongylus (14.8 and 4.8%), Coccidia (15.7 and 9.7%), paramphistomum (14.5 and 9.7%), Ostertagia (1.7 and 0%), Haemonchus (1.2 and 0%), Trichuris (0.3 and 0%) and mixed infection (17.5 and 17%) were recorded in goats and sheep, respectively.

Furthermore, the prevalence of Oesophagastamum (0 and 2.4% and Fasciola (9.7 and 11.7%) were recorded in sheep and goats, respectively which were the only genus recorded more prevalently in sheep than goats (Table 3). Regarding the relationship of age with specific species of parasite; Haemonchus, Paramphistosomum, Coccidia, Oesophagastamum and mixed infection were higher in young; whereas Fasciola, Strongloides, Ostertagia, Trichostrongylus, and Trichuris were higher in adult animals (Table 3). Regarding the association of sex with GIT parasite of small ruminant the study revealed that males were 1.409 times more likely to be positive for GIT females. However, this variation were not statically significant during multivariable logistic regression analysis (OR=1.409, CI=0.745-2.663, *P*= 0.291). The parasite than prevalence of Fasciola, Paramphistosomum, Trichuris, Ostertagia, Strongloides, Trichostrongylus species and mixed infection were higher in female than males in both animals (Table 3).

DISCUSSION

The present study revealed the overall GIT parasite prevalence of 77.8% (299/384) with 79.6%(273/343) and 63.4%(26/41) in goats and sheep, respectively. This result coincides with the results of previous studies reported from different parts of Ethiopia which includes prevalence of 76.03% from Welinchity, Central Ethiopia (Moti, 2008) and79.09% from Debre Berhan, Northern Ethiopia (Achenef, 1997). The result of current study was higher than the result of different scholars. For instance, Arsi Negele (Central Ethiopia) with prevalence of 69.01% (Dilgasa et al., 2015), Haromaya town (South Eastern Ethiopia) with prevalence of 70.2% (Berisa et al., 2011), Gonder town (Northern Ethiopia) with prevalence of 70.8% (Yimer and Birhan, 2016), Walanchity (Central Ethiopia). However, the prevalence found by this study was lower than that reported from Wolayita Soddo, 86% (Dereje, 1992); Illubabor, 91.4% (Melkamu, 1991). This variation could be due to the difference in agro-ecology of area of study, season of study, sample size, methods of examination employed, flock size, management system and deworming activities performed in respective areas. In this study, there was statistically significant difference (p=0.010) in prevalence between species of animals as goats were 2.821 times more likely to be positive for GIT parasite than sheep. This result coincides with (Abebe and Esayas, 2001) and could be due to higher immune response of sheep to GI parasites than goats and the habit of mixed flock, in which sheep are relatively passive and usually graze/browse from back of the flock following more alert and voracious mass of goats in front line that may get access to more feedstuff and parasites as well.

Table 3. Prevalence of different genera of parasites in relation to species, age and sex of study animals.

		Fasciola	Strongloides	Haemonchus	Trichostrongylus	Coccidian	Ostertagia	Trichuris	Paraphistomum	Oesophagostomum	Mixed infection
Species	Caprine	9.7	25.36	1.1	14.8	15.7	1.7	0.3	14.5	0	17.5
	Ovine	11.7	24.4	0	4.8	9.7	0	0	9.7	2.4	17
	Overall	11.5	25.26	10.4	13.8	15.1	1.5	0.26	14	0.26	17
Age	Adult	12.6	28.3	0.4	15.6	10.8	2.2	0.8	11.7	0	11.7
	Young	9.7	20.1	1.9	11	21.4	0.6	0.6	17.5	0.6	26
Sex	Male	10.3	19.5	1.1	8	31	0	3.4	11.5	1.1	15
	Female	11.5	26.6	1	15.4	10.4	2	0	14.8	0	18

Table 4. Degree of parasite infestation in study animals.

Degree of infestation	Caprine n (Prevalence)	Ovine n (Prevalence)	Overall Prevalence (%)
Heavy	85(31.1%)	10(38.5%)	92(31.8%)
Moderate	84(30.7%)	6(23%)	90(30.1%)
Light	104(38%)	8(30.7%)	112(37.5%)
Total	273(79.6%)	26(63.4%)	299(77.8%)

n= Number of positive animals.

Table 5. Prevalence of grouped parasite among districts.

District	Nematode	Trematode	Eimeria	Overall
MaddaWalabu	64(58.7%)	31(28.4%)	14(12.8%)	**109(81.9 %)**
Harana Buluk	48(53.3%)	25(27.8%)	17(18.8%)	**90(76.9%)**
Dallo Manna	45(45%)	32(32%)	23(23%)	**100(74.6%)**
Overall	**157(40.8%)**	**88(23%)**	**54(14%)**	**299(77.8%)**

Regarding the prevalence of identical genera of sheep and goats are heterogeneously infested. The prevalence of identified parasites was higher in goats than sheep except Fasciola and Oesophagostomum which may account for the species susceptibility and resistance to the specific parasite. Additionally, unlike goats, sheep are grazers and may harbor Fasciola with contaminated pastures or by grazing around swampy area.

This study also revealed significant difference in prevalence of GIT parasite among different body condition scores (P=0.004). Animal with good body condition was more likely prone to the GIT parasites disagreeing with (Abebe and Esayas, 2001; Tefera et al., 2009) but we justify this that emaciation observed in studied animals might be due to malnutrition, concurrent infections and/or

Table 6. Prevalence of grouped parasite among species, sex and age of animals.

Parameters		Nematode	Trematode	Eimeria
Species	Caprine	143(41.6%)	81(23.6%)	49(14.3%)
	Ovine	14(34.1%)	7(17%)	5(12%)
	Overall	**157(40.8%)**	**88(23%)**	**54(14%)**
Sex	Male	26(30%)	18(20.6%)	25(28.7%)
	Female	130(43.7%)	69(23.2%)	31(10.4%)
Age	Adult	106(45.8%)	48(20.7%)	30(13%)
	Young	51(33.3%)	40(26.1%)	24(15.6%)

Table 7. Degree of infestation for grouped parasites.

Degree of infestation	Nematode	Trematode	Eimeria	Overall
Light	54(34.4%)	32(36.4%)	22(40.7%)	**108(36%)**
Moderate	42(26.7%)	26(29.5%)	22(40.7%)	**85(28.4%)**
Heavy	61(38.8%)	30(34.1%)	10(18.6%)	**92(30.7%)**
Overall	**157(40.8%)**	**88(23%)**	**54(14%)**	**299(77.8%)**

since more than 77% of studied animals were female lowered body condition could be due to lactation and post kidding stresses. In addition, development of an acquired immunity after prolonged exposure may obscure manifestation of clinical signs in infested animal.

Regarding association of sex with GIT parasite of small ruminant the study revealed that males were 1.409 times more likely to be positive for GIT parasite than females. However, this variation were not statically significant during multivariable logistic regression analysis (*P*= 0.291). This result agrees with (Tefera et al., 2009; Fikru et al., 2009) who showed that there is no significant association between sex of animal and prevalence of GIT parasites in small ruminants and revealed that both sexes have equal chance to get infection equally as both male and female animals are kept under similar management system. However, at the generic level the prevalence of Fasciola, paramphistosomum, Trichuris, Ostertagia, Strongloides, Trichostrongylus species and mixed infection were higher in female than males in both animals. Higher prevalence of these parasites in female animals agrees with (Emiru et al., 2013; Owusu et al., 2016) and may be due to stress and reduced immune status during pregnancy, parturient paresis and lactation periods.

The current study showed the statistically significant variation in overall prevalence of GIT parasite in age group. Adult animals were 2.19 more likely to be prone to GIT parasite than young animals. This result is in agreement with (Emiru et al., 2013). Furthermore, at level of specific species of parasite, the prevalence of Haemonchus, Paramphistomum, Coccidia, Oesophagastamum and mixed infections were higher in young; whereas Fasciola, Strongloides, Ostertagia, Trichostrongylus, and Trichuris were higher in adult animals. This result agrees with Emiru et al. (2013) and Admasu and Nurlign (2014). This variation in susceptibility could be due to nutritional factor, immune status, management system and the frequency of exposure to the respective parasite.

Regarding GIT parasite prevalence distribution among districts, the highest prevalence was recorded in Madda Walabu (81.9%) followed by Haranna Buluk (76.9%) and Dallo Manna district (74.6%). However, this variation was not statistically significant during multivariable analysis (p=0.139). At Peasant association level, parasite prevalence is highest in Ella Bidire Peasant association (97.7%) while lowest prevalence was that of Kumbi Peasant association (57.1%). Difference in agro-climate, management system and veterinary service in the respective districts and Peasant associations might attribute to the variations of prevalence recorded.

Conclusion

Present study revealed that polyparasitism is a major health problem and hindrance in small ruminants' production in current study area due to high prevalence (77.8%).

A burden of gastrointestinal parasite is distributed in heterogeneous manner among districts and respective Peasant associations; indicating uneven delivery of veterinary service and unsound husbandry and management practice among districts and Peasant associations. Therefore, periodic and strategic deworming intervention with effective broad spectrum anti-helminths encompassing all localities of the study area is needed to mitigate this daunting problem. In addition, proper grazing system and stocking size should be practiced. Furthermore, awareness of the society toward GIT parasite has also paramount importance in prevention and control measures. Moreover, further studies are also recommended to identify the more genus and species of GIT parasites in this area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Prevalence of Cryptosporidiosis infection among children under 5- years in Cotonou, Benin

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Cryptosporidiosis is recognized as one of the leading causes of childhood diarrhea in developing countries, but no data has been published, so far, on the prevalence among children with diarrhea in Benin. The aim of the present study was to assess, for the first time, the prevalence of *Cryptosporidium* spp. infection in Cotonou, Benin. A prospective study involving children younger than 5 years of age (n = 104) hospitalized or consulted in the paediatric departments of three hospitals between September 2015 and May 2016 was carried out. *Cryptosporidium* oocysts were detected by means of a smear of sedimented stool stained by the modified Ziehl-Neelsen technique and were found in 5.8% of the studied population. Neither age, non-use of drinking water, nor nutritional status influenced the presence of cryptosporidiosis in the study, but 5 of the 6 children infected were less than two years of age. In conclusion, this first study showed that infection with *Cryptosporidium* spp. could be a cause of diarrhea in a not insignificant proportion in Cotonou. Studies with larger numbers of patients, conducted for a longer time and in rural areas would be necessary to estimate the real prevalence of cryptosporidiosis and to assess the risk factors.

Key words: Cryptosporidiosis, prevalence, children, Benin.

INTRODUCTION

Diarrheal disease, a leading cause of mortality and morbidity in young children, is estimated to cause more than 760000 annual deaths among children of < 5 years of age (WHO, 1986), with 72% of these deaths occurring

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in children of < 2 years of age (Walker et al., 2013). Sub-Saharan Africa accounts for half of all global childhood deaths from diarrheal diseases (Walker et al., 2013). In Benin, according to health statistics (Ministry of Health, 2014), diarrhea is responsible for 3.1% of hospitalizations. Diarrheal diseases can be caused by various bacteria, viruses and parasites. Among the parasitic causes, cryptosporidiosis is recognized as one of the leading causes of childhood diarrhea in developing countries (Davis et al., 2002; Fletcher et al., 2012). Initially recognized in highly immunocompromised people infected with HIV, cryptosporidiosis may also be of major public health importance in young children. It has dramatic adverse effects on child growth and development and causes increased mortality in developing countries where HIV, poverty, and lack of sanitation and infrastructure increase the risk of cryptosporidial waterborne infection. Transmission occurs via the fecal-oral route from human and animal reservoirs. The symptoms of acute cryptosporidiosis include severe watery diarrhea, eventually leading to dehydration, malabsorption and malnutrition. In immunocompetent hosts, cryptosporidiosis is usually self-limiting, but in developing countries, it contributes to persistent diarrhea in children (Huang et al., 2006). Thus, studies conducted in sub-Saharan Africa among young children showed prevalence that vary from one country to another and may reach 35% for *Cryptosporidium* spp. (Kassi et al., 2004; Gay-Andrieu et al., 2007; Mbae et al., 2013; Tellevik et al., 2015; Nassar et al., 2016). However, the burden of cryptosporidiosis, can be underestimated due to the presence of many silent asymptomatic carriers and poor performance of traditional diagnostic procedures in many laboratories which can result in misdiagnosis and mistreatment with serious and possibly fatal outcomes, especially in young children. In Benin, the prevalence of cryptosporidiosis in HIV infected people is reported as 10.8% (Loko et al., 2008). However, this pathogen is not often sought as etiology during diarrhea in children in Benin and no information is available on the prevalence of cryptosporidiosis in children. The present study investigated the prevalence of *Cryptosporidium* infection among young children with diarrhea in Benin and attempted to identify risk factors for infection. The results will be used as a database in the orientation of the management of diarrhea in children.

MATERIALS AND METHODS

Study site, sample and data collection

The study was performed between September 2015 and May 2016, primarily during the dry season, in three hospitals, namely the Centre National Hospitalier et Universitaire Hubert Koutoucou MAGA (CNHU/HKM), the Centre Hospitalier Universitaire de la mère et de l'enfantlagune (CHUMEL) and the Mènontin district hospital, all located in Cotonou in the south of Benin. Laboratory analyses were carried out in the Laboratory of Parasitology of CNHU/HKM. The study was conducted among children < 5 years of age admitted with diarrhea in one of the three hospitals involved. Written informed consent was obtained from one of the child's parents. Diarrhea was defined as three or more watery stools within 24 h. An episode of diarrhea was considered over when two consecutive days passed without diarrhea. An episode of acute diarrhea was defined as lasting between 24 h and less than 14 days. Persistent diarrhea was defined as diarrhea for 14 days or more. Children were categorized as having normal nutritional status, or either mild or severe malnutrition using weight -for length Z score according to WHO criteria (WHO, 1986). The HIV status of each child was sought in the registry. HIV tests were conducted for those for whom the status was not known. All children admitted for paediatric consultation or who were hospitalized, and who met the inclusion criteria, were tested for oocysts of cryptosopridia in a stool specimen that was collected and sent to the Parasitology Laboratory of the CNHU. For sites other than the CNHU, the stool samples were kept at 4°C prior to transportation in cold boxes. A standardized questionnaire was used for collection of demographic and clinical information. In the laboratory, direct stool examination and Ritchie technique were systematically performed, and the presence of *Cryptosporidium* oocysts was assessed by means of a smear from the sediment which was stained by the modified Ziehl-Neelsen technique (Deluol, 1999). A double reading was made for each smear by experienced technicians of the parasitology laboratory of CNHU, the national reference centre for parasitology. In case of discrepancy, a third reading was made by another technician for the validation of the final result. Samples having red oocysts of 4 to 5 μm in diameter were considered positive for *Cryptosporidium* infection.

Data analysis

Data were handled and analyzed using Excel software and Epi Info 2000 software. Qualitative variables were described in terms of number and percentage. For quantitative variables, averages and standard deviations were calculated. Proportional comparisons were made using the Chi-square test, with $P < 0.05$ being considered statistically significant.

Ethics statement

The Ethical Committee of the School of Medicine and Health Sciences, University of Benin, gave the ethical approval for the study. The study was approved by the respective hospital authorities at the three study hospitals. Written informed consent was obtained from the parents or guardian of all the children enrolled in the study.

RESULTS

A total of 104 children of less than 5 years of age were recruited into the study, where the mean of ages was 15 ± 12.03 month. The female sex was the most represented and the majority of the children had a normal nutritional status. Only three of the study children attended school, and more than half of their parents had a primary level of education. Majority of the children used drinking water and latrines: 82.7% and 81.7% respectively (Table 1). All the children were HIV negative. With regards to clinical symptoms, there was fever in most of the cases as well as vomiting, while dehydration and abdominal pain were found in smaller proportions

Table 1. Some socio-demographic variables related to children (n = 104) examined for crypotosporidiosis in Cotonou, Benin

Variable	Number examined (%)
Age in years	
<2	86(82.7)
2-5	18(17.3)
Sex	
Male	51(49)
Female	53(51)
Ratio weight for length	
Normal nutritional status	86(82.7)
Moderate malnutrition	6 (5.8)
Severe malnutrition	5(4.8)
Overweight	7(6.7)
Schooling of children	
Yes	3(2.9)
No	101(97.1)
Parent level of education	
Primary school	60(57.7)
Secondary school	33(31.7)
University	11(10.6)
Use of drinking water	
Yes	86 (82.7)
No	18(17.3)
Use of latrine	
Yes	85 (81.7)
No	19(18.3)

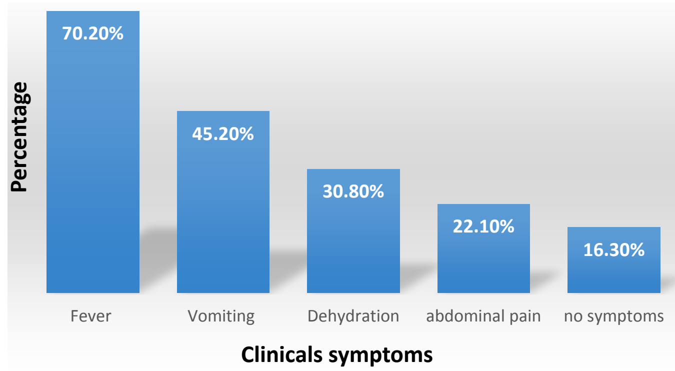

Figure 1. Clinical symptoms associated with diarrhea among children (n = 104) of Cotonou, Benin.

Table 2. Characteristics of stool collected from children (n = 104) in Cotonou, Benin.

Variable	Number examined (%)
Evolution of diarrhea	
Acute	91 (87.5)
Chronic	13 (12.5)
Number of stools/per day	
≤ 5	73 (70.2)
>5	31(29.8)
Stool consistency	
Liquid	56(53.8)
Semi-liquid	48(46.2)

(Figure 1). Table 2 shows the characteristics of diarrhea, where the majority of children had acute diarrhea and 70.2% had less than 5 stools per day. Of the 104 children examined, 6 had oocysts of *Cryptosporidium* spp. in their stool, giving a prevalence of 5.8%. Neither age, non-use of drinking water, nor nutritional status influenced the occurrence of cryptosporidiosis in this study (Table 3).

DISCUSSION

During the study period, 104 children of less than five years with diarrhea were recruited, a smaller study population as compared to studies in Niger and Senegal (Gay-Andrieu et al., 2007; Faye et al., 2013). The difference could be explained by the fact that in Senegal, the study focused on children aged 0 to 15 years, while in Niger, both diarrheal and non-diarrheal children were concerned. The predominance of children under 2 years of age in the study population could be explained by the fact that this is an age group more vulnerable to diarrhea because basic hygiene rules are neither known nor respected. This high proportion of children under 2 years of age also explains why very few children in our study attended school, because the age of schooling in Benin is generally around 3 years. Identifying school attendance for children relates to the fact that the peak of parasitism occurs at the age at which children are sent to kindergarten and primary schools when promiscuity, community games and contact with dirty soil favour contamination. Moreover, the rate of malnourished children in this study population (10.6%) is lower than the rate found at the national level, which was 36% for children less than 5 years (unpublished data from the Ministry of Health). This is probably due to the city of Cotonou being the site of our study, the economic capital of Benin, where children are better fed than in rural areas where malnutrition rates could be higher (INSAE, 2012).

The prevalence of cryptosporidia oocysts in the study population was 5.8%. This comparatively low prevalence

Table 3. Some risk factors and cryptosporidiosis infection among children (n = 104) of Cotonou, Benin (n = number infected; N = number examined; % = prevalence of infection; RP = prevalence report).

	N	n	%	RP [IC $_{95\%}$]	p.value
Age in years					
<2	86	5	5.8	1.05 [0.01-10.00]	0.966
2-5	18	1	5.6		
Use of drinking water					
Yes	86	5	5.8	0.95 [0.88-10.15]	0.966
No	18	1	5.6		
Nutritional status					
Normal	86	4	4.7	4.1 [0.75-22.45]	0.824
Moderate malnutrition	12	2	16.7		

could be explained in several ways. The study was conducted in Cotonou, a city where the prevalence of intestinal parasitic infections has decreased due to improved access to drinking water and latrine use, which reduces fecal contamination rates, thus limiting occurrence of parasitosis in general and cryptosporidiosis in particular. Cryptosporidiosis is a disease related to hydrofecal contamination, which justifies exploring the study population's habits with regard to the use of drinking water. The small size of our sample due to the study period may also explain this low prevalence. Indeed, this study was conducted largely in the dry season which characterized by low rainfall, a period thus not favoring the multiplication of oocysts. Studies have shown that the prevalence of cryptosporidiosis is greater in the rainy season (Duong et al., 1995; Tumwine et al., 2003; Jagai et al., 2009; Siwila et al., 2011; Mbae et al., 2013; Tellevik et al., 2015), the rains causing the dissemination of fecal matter that contaminates drinking water, fruit and vegetables. This low prevalence could be due to methodology, using microscopy which can be less sensitive than PCR, that we were unable to take account because of limited financial resources. A study of a larger size conducted in the rainy season, and taking into account rural environments, could lead to stronger conclusions regarding the prevalence of cryptosporidiosis in children in Benin, because taking into account all the above, the figure of 5.8% reported here could clearly be a low estimate of overall prevalence. Significant variation between generally more affected rural environments compared with urban environments has been noted by others (Lu et al., 2008). Health authorities will need to sensitize medical laboratories to the need to examine stools for oocysts of *Cryptosporidium* spp., a simple and inexpensive exercise, in order to improve the management of diarrhea in children under 5 years of age. Hospital-based diagnostic laboratories in Benin currently do not routinely look for this parasite. It should be noted that infection with *Cryptosporidium*s spp. as a direct cause of diarrhea in the children in this study is important to consider because no other parasitic infection was detected. Kassi et al. (2004) in Abidjan found that in 80% of carriers of *Cryptosporidium* spp., it was the only parasitic infection found. Similarly, Hojling et al. (1984) reported that, in carriers of cryptosporidia, it is often a mono-infection. The prevalence reported here is close to the 5.5% found by Gay-Andrieu et al. (2007) in Niger, the 5.6% found by Areeshi et al. (2008) in Madagascar and the 4.5% reported by Faye et al. (2013) in Senegal. On the other hand, it is lower than that found in Abidjan, Tanzania, Ethiopia and Rwanda (Kassi et al., 2004; Kabayiza et al., 2014; Tellevik et al., 2015; Wegayehu et al., 2016), respectively 7.7, 10.4, 9.4 and 7.2%. In the Kassi et al. (2004) study in Abidjan, 18.5% of the children included were infected with HIV, while in Tanzania, the study was conducted only in children under 2 years of age and from August to July, a rainy season favouring the spread of oocysts. High prevalence have also been found in countries with high average rainfall such as Gabon and Nigeria (Duong et al., 1995; Nassar et al., 2016), with 31.6 and 38.3%, respectively.

The low prevalence of cryptosporidiosis in this study may explain the absence of risk factors for cryptosporidiosis. Age was not a risk factor; although, 5 out of 6 children who had cryptosporidiosis were less than two years of age, suggesting that cryptosporidiosis is more common in young children. This would be consistent with the results of several studies showing that cryptosporidiosis affects infants more frequently (Dieng et al., 1994; Perch et al., 2001; Gatei et al., 2006; Khalili and

Mardani, 2009; Mbae et al., 2013; Kotloff et al., 2013). Not using drinking water also does not appear to be related to the occurrence of cryptosporidiosis. The same observation was made by Sakar et al. (2013) in India, in a study aimed at verifying the risk of using municipal water sources and bottled water in the occurrence of cryptosporidiosis. Other studies have nevertheless shown the involvement of water type in the occurrence of cryptosporidiosis. This is the case in the epidemic in Milwaukee caused by contamination of the city's water system (MacKenzie et al., 1994) and a study in Sudan that showed a higher prevalence of cryptosporidiosis in residents of rural areas who do not have access to safe drinking water (Sim et al., 2015). These findings suggest that the use of water purification systems is important for preventing *Cryptosporidium* infection among inhabitants of rural areas. All children with cryptosporidiosis in the study population were seronegative for HIV. Studies in Tanzania, Kenya and Uganda found that infection with *Cryptosporidium* was found more frequently in HIV-infected children (Tumwine et al., 2005; Mbae et al., 2013; Tellevik et al., 2015).

However, *Cryptosporidium* should not be ignored as a cause of diarrhea in small children not known to be HIV-positive, as the GEMS-study found that it was an important pathogen at all sites regardless of HIV sero-prevalence, and the second most common pathogen causing diarrhea in infants (Lu et al., 2008). A relationship between malnutrition and cryptosporidiosis was also not found in this study due to the very few malnourished children. On the other hand, Gay-Andrieu et al. (2007) in Niger found a high proportion of malnourished children among children with cryptosporidia (10/12 or 83%). Also, Tellevick et al. (2015) in Tanzania showed that children who had stunted growth had a significantly higher risk of being infected with *Cryptosporidium*. Molloy et al. (2011) and Yones et al. (2015) also found association between stunting and *Cryptosporidium* infection among Nigerian and Egyptian children respectively, which supports the findings that stunted children had a significantly higher risk of being infected with *Cryptosporidium*.

Conclusion

This first study, carried out in children less than five years of age with diarrhea, showed that the prevalence of cryptosporiodiosis was estimated at 5.8%, a possible underestimation. A study with a larger sample size and of a longer duration is needed not only to determine the real prevalence of this parasitosis but also to assess the associated risk factors in Benin.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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