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Ovine Eimeria infection, OPG and determinants in and around Gondar town, Ethiopia

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ABSTRACT

The study aimed to assess the prevalence of ovine *Eimeria* infection, assess the putative risk factors, and the intensity of infection. A cross-sectional study design was employed and the study was carried out from November 2017 to May 2018. A total of 422 sheep were selected by systematic random sampling technique, and from these animals, faecal samples were collected and examined for *Eimeria* oocysts. Of the selected and examined sheep 132 (31.3%, 95% CI=26.8-35.7) were found infected by *Eimeria* species. Eimeria infection prevalence was significant (P <0.05) higher in lambs/young than the adult, in females than males, and in poor body condition than in medium body condition sheep. Moreover, the prevalence of Eimeria infection was significantly (P <0.05) higher in sheep with soft faeces than in normal faeces sheep and semi-intensive than extensive sheep production. The intensity of *Eimeria* species infection was influenced by the age, sex, body condition, fecal consistency, and production system of sheep (P< 0.05). The overall mean Oocysts per gram of faeces was 2390.6 (95% CI=2007.5-2773.8). The mean OPG was significantly higher in lambs, with poor body conditions and female sheep. In addition, it was higher in sheep with soft faeces and an extensive production system. In conclusion *Eimeria* species infection was an important problem of sheep production in the study area. Generally, this study's results provided useful information to design and implement appropriate control strategies. Finally, it is recommended that further study identify the species of *Eimeria* circulating in the areas.

INTRODUCTION

Ethiopia is home to a large and diverse livestock resource (Gizaw *et al*., 2010); and the country has around 17 million sheep (CSA, 2021). This means sheep represent an important segment of the livestock system in the country. Sheep are important sources of income for the agricultural communities, represent one of the country's major sources of foreign currency through the export of skins and meat, and are a source of animal protein. They also play a major role in the food supply and social well-being of rural communities living in conditions of extreme poverty, which is the particular case in parts of Ethiopia (Dagnew *et al*., 2017; Gizachew *et al*., 2014). The major constraints for sheep production in Ethiopia are feed shortage, grazing land limitation, problems related to Veterinary Services, and diseases (Welday *et al*., 2019; Kenfo *et al*., 2018; Nigussie *et al*., 2013).

According to Gizaaw *et al*. (2013), one of the research gaps is sheep diseases, of which

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parasites are among the most important diseases. Among parasitic diseases, Coccidiosis is an important protozoan parasitic infection that is responsible for low productivity, impaired growth, and mortality in sheep populations (Ayana *et al*., 2009). It is caused by a protozoan parasite of the genus *Eimeria* that affects young animals in particular (Hendson and Agnes, 2002). They are highly species-specific, meaning the species of *Eimeria* that infect sheep will not infect goats or cattle and vice versa (Constable *et al*., 2017; Aitken, 2007). Coccidiosis in sheep was reported in various parts of the world (Khan *et al*., 2011; Bukar, 2007; Toulah, 2007). Also reported from Ethiopia (Lakew and Seyoum, 2016; Ayana *et al*., 2009), but in the country, there are some areas with limited information about the prevalence of *Eimeria* species infection in the sheep. There is no study of *Eimeria* species infection prevalence of sheep in and around Gondar. Therefore, the aims of this particular study were to estimate the prevalence of *Eimeria* species infection, to identify the putative risk factors and to assess the intensity of infection in sheep in and around Gondar.

MATERIALS AND METHODS

Study Area

This study was carried out in and around Gondar town (Azezo, Tseda, Maksegnit, Angereb, Ayra and Fenter Kebeles) from November 2017 to May 2018. Gondar town is located in Amhara regional State, North-West Ethiopia. The study area is located at an latitude and longitude of 12.30 - 13.80 °N, 35.30 - 35.7°E; and its altitude is on average about 2220 meters above sea level. The mean annual rainfall and temperature of the area were 1172mm and 19.7ºC, respectively (National Meteorological Agency, 2017).

Study animals

The study animals of the study were sheep flock reared under an extensive and semi-intensive management system. All grazing age groups, local breed,, and both sexes of sheep were considered for the study (Breed information was obtained with personal communication with the owners).

Study design

Across sectional study design was employed to estimate the prevalence of *Eimeria* infection of sheep in and around Gondar town. During this study faecal consistency, age, sex and body condition of the sheep, the hygienic status of the animal house and management system were considered as potential risk factors (independent variables) for *Eimeria* infection (dependent variable) of sheep. The study animals were divided into three age groups, namely: lamb (less than 6 months old), young (6-12 months old), and adult (over 12 months old) as described by ESGPIP (2009) and Gatenby *et al*. (1991). Faecal consistency was examined and classified as normal (Formed pellets), soft (Pellets not formed) as described by (Platzer *et al.,* 2005). Body conditions of the study animals were scored as poor, medium, and good as described by Russel (1991).

Sample size determination & sampling techniques

The required sample size for this study was computed by considering 50% prevalence of ovine *Eimeria* spp. infection, to obtain the

maximum sample size. The formula described by Thrusfield (2018) was used to compute the sample size required for the study. The study considered a 95% confidence interval and 5% absolute precision. Therefore, 384 sheep were required for the study; but 422 animals were selected and studied. A systematic random sampling method was used to select the study animals from the target sheep flock.

Study methodology

Faecal sample collection

Faecal samples, about 10gm, were collected directly from the rectum of the sheep by using arm-length plastic gloves, and placed in screw capped universal sample bottle. Each samples was labeled with the necessary information about the animal and the sample (i.e. age, sex, faecal consistency and body condition score) were recorded on the format prepared for this study. Moreover, the hygienic status of the animal and the management system was carefully observed. Finally, the samples were packed in icebox with ice packs and transported to the parasitological laboratory of the Faculty of Veterinary Medicine, Gondar University. The samples were kept in refrigerator at 4ºC until examined. Collected samples were examined within 24 hours for the presence or absence of Eimeria oocysts as described previously (Taylor *et al*. 2016; Zajac and Conboy, 2011).

Qualitative and Quantitative faecal examination

From the collected faecal samples, 3gm was weighed and properly mixed with 42ml flotation fluid (Saturated salt solution). Then sieved by tea strainer and filled to test tube, and then centrifuged at 1,500 rpm for 3 minutes. The sample taken out of the centrifuge and the supernatant was examined for Eimeria oocysts under 10X and 40X microscopic magnifications as described by Urquhart et al. (1996). Those faecal samples positive in the qualitative examination were subjected to quantitative examination. For this examination, McMaster egg counting technique (Urquhart *et al*., 1996; MAFF, 1986) was used to determine the number of oocysts per gram of faeces and assess the intensity of infection.

Data management and analysis

Collected data were entered into a Microsoft Excel spreadsheet, cleaned, coded, and then summarized by descriptive statistics like percentages or proportion and mean. The prevalence of *Eimeria* infection was computed by dividing the number of positive animals by the total number of animals examined and multiplied by 100. The association of the various risk factors considered for this study was analyzed by univariable logistic regression analysis. Those non-collinear factors with a p-value of ≤ 0.25 in the univariable logistic regression analysis were further analyzed by multivariable logistic regression analysis. The dependability of the fitted model was further evaluated using the receiver operating characteristic curve (ROC). Finally, the model fitness was assessed by the Hosmer-Lemeshow goodness fit test as described by Dohoo *et al*. (2009). The mean number of oocysts per gram of faeces was computed by considering those sheep positive in the qualitative examination for oocysts. Oocysts per gram of faeces was determined and then after, to normalize the data were log-transformed ($\log_{10+1}$). The transformed data were analyzed by using a t-test for the existence of associations between oocysts per

gram of faeces (OPG) and the risk factors were analyzed by paired t-test. For the data analysis, STATA 14.2 software (Stata Corp 4905 Lakeway Drive, College Station, Texas 77845 USA) was used.

RESULTS

Qualitative faecal examination results

From a total of 422 examined sheep 132 (31.3%, 95% CI=26.8-35.7) were infected by *Eimeria* species. The association of risk factors for infection of sheep by *Eimeria* species was shown in table 1.

Table- 1: Results of Logistic regression analysis of risk factors for Eimeria infection in sheep in and around Gondar

Variable	Category	No. examined	Prevalence		Univariable		Multivariable		
			No. (%) positive	95% CI	OR	P-value	OR	95% CI	P-value
Age	Lamb	96	56 (58.3)	48.4-68.3	6.3	≤0.001	5.4	3.2-11.9	≤0.001
	Young	151	44 (29.1)	21.8-36.4	1.8	0. 022	2.8	1.3-4.2	0.005
	Adult	175	32 (18.3)	12.5-24.0	Rf.	-	Rf	-	-
Sex	Male	237	96 (19.5)	13.7-25.2	Rf.	-	Rf	-	-
	Female	185	36 (40.5)	34.2-46.8	2.8	≤0.001	3.8	1.7-4.9	≤0.001
Faecal consistency	Normal	242	56 (23.1)	17.8-28.4	Rf.	-	Rf.	-	-
	Soft	180	76 (42.2)	35.0-49.5	2.4	≤0.001	5.7	2.6-7.1	≤0.001
BCS	Poor	147	73 (49.7)	41.5-57.8	3.6	≤0.001	2.2	1.1-3.0	0.030
	Medium	275	59 (21.5)	16.6-23.3	Rf.	-	Rf	-	-
Management system	Extensive	244	43 (17.6)	12.8-22.4	Rf.	-	Rf	-	-
	Semi-intensive	178	89 (50.0)	42.6-57.4	4.7	≤0.001	5.7	2.6-7.1	≤0.001
Total		422	132 (31.3)	26.8-35.7		-	-	-	-

BCS= Body condition; NB. OR= Odds ratio; CI= Confidence interval; Rf=Reference category

All the independent variables were non-collinear [gamma value (γ) value fall between -0.6 and +0.6], and the univariable analysis p-value was <0.25. Hence, all the risk factors were subjected to multivariable analysis. The final model had Hosmer-Lemeshow χ^2 (8) = 13.66, P= 0.091, and ROC= 0.7621 that there is no significant difference between the observed and predicted values.

Quantitative faecal examination result

The minimum and maximum numbers of Oocysts per gram of faeces (OPG) were 300 and 12,500, respectively. The overall mean OPG of faeces was 2390.6 Oocysts/gram of faeces (Table 2).

Table- 2: Mean OPG and t-test analysis of risk factors for intensity of *Eimeria* species infection

Risk factors and its levels		No examined	No positive	Mean OPG	95% CI	t-value	P-value
Sex	*Female	237	96	2618.9	2146.4-3091.3	4.95	≤0.001
	*Male	185	36	1781.9	1172.0-2391.9	Rf.	
Age	-Lamb	96	56	3294.8	2638.3-3951.3	8.01	≤0.001
	-Young	151	44	1920.5	1324.7-2516.2	2.44	0.015
	-Adult	175	32	1454.7	914.8-1994.6	Rf.	
Body condition	*Poor	147	73	3266.6	2714.7-3818.4	7.12	≤0.001
	*Medium	275	59	1306.8	931.1-1682.5	Rf.	
Faecal consistency	-Soft	180	76	3008.7	2462.5-3554.9	4.84	≤0.001
	-Normal	242	56	1551.8	1107.0-1996,6	Rf.	
Management system	*Extensive	244	43	2061.6	1421.5-2701.8	Rf	
	*Semi-intensive	178	89	2549.6	2067.9-3031.2	7.62	≤0.001
Overall		**422**	**132**	**2390.6**	**2007.5-2773.8**		

DISCUSSION AND CONCLUSIONS

The prevalence of *Eimeria* species infection of sheep in and around Gondar was 31.3%. All risk factors considered during this study for *Eimeria* species infection of sheep were significantly ($p< 0.05$) influencing infection of sheep by *Eimeria* species. Lamb and young sheep were 5.4 and 2.8 times more likely to be exposed to *Eimeria* species infection than the adult sheep, respectively. Similar observations were reported from various areas of the world (Elkhatam *et al*., 2020; Etsay *et al*., 2020; Paul *et al*., 2020; Kiltuet al., 2016; Nourollahi-Fard *et al*., 2016; Lakew and Seyoum, 2016; Wang *et al*., 2010; Maingi and Munyua, 1994; Kanyari, 1993). Infection of sheep followed by the development of species-specific immunity, and so, sheep infected and immune to one species of *Eimeria* may not be re-infected again by the same species of *Eimeria*. This difference might be related to earlier infection and the development of immunity in adult sheep (Matos *et al*., 2018; Constable *et al*., 2017; Rehman *et al*., 2011; Yakhchali and Golami, 2008). The other justification could be there is no active immunity to *Eimeria* species in younger naïve sheep (Paul *et al*., 2020).

This study showed that *Eimeria* infection was significantly ($P< 0.05$) higher in female sheep than in male sheep as also reported by various authors (Elkhatam *et al*., 2020; Etsay *et al*., 2020; Paul *et al*., 2020; Sharma *et al*., 2017; Kiltu *et al*., 2016; Khan *et al*., 2011; Rehman *et al*., 2011; Sharma *et al*., 2009; Yakhchali and Golami, 2008), which reported sex influences the prevalence of ovine Coccidiosis. This might be attributed to sex-related factors including the physiological stress encountered by the ewe (i.e. pregnancy; lambing, and suckling the newborn lamb), which was the reason for the ewe being more susceptible to *Eimeria* infections (Heidari *et al*., 2014; Lopes *et al*., 2013).

A significantly higher ($P< 0.05$) prevalence of *Eimeria* infection was found in semi-intensive than extensive production systems of sheep, which is also reported in various areas of the

world (de Macedo *et al*., 2020; Lakew and Seyoum, 2016; Kanyari, 1993). In an extensive management system, animals are freely moving in larger areas, and hence, the chance of infection is decreased as compared to the semi-intensive management system. It is known that a semi-intensive management system (i.e. where high animal population density occurs) contributed to the propagation of *Eimeria* species (de Macedo *et al*., 2020). As the flock size of sheep increases, there is greater contamination of feeding and watering troughs (Yakhchali and Rezaei, 2014). Hence, confinement was found to contribute to high prevalence (Kanyari, 1993).

In this study, a significant association was documented between body condition score and *Eimeria* infection. Similarly, Lakew and Seyoum (2016) and Khan et al. (2011) reported a higher infection rate in sheep with poor body condition scores. The poor body condition in sheep might be due to the immunosuppressive effect of concurrent disease problems and/or nutritional scarcity. All these can negatively influence the animal feed intake and weaken the immunity, and the sheep become highly susceptible and get infected (Constable et al., 2017).

During this study, a significant ($P< 0.05$) association of *Eimeria* species infection was observed in sheep with soft faecal consistency than the normal faeces. This finding is in a general agreement with reports from various parts of the world (Khodakaram-Tafti and Hashemnia, 2017; Lakew and Seyoum, 2016; Yakhchali and Rezaei, 2014; Yakhchali and Golami, 2008). Infection by most of the pathogenic *Eimeria* species leads to the destruction of intestinal epithelial cells and induces enteritis followed by diarrhea (Constable *et al*., 2017; Chartier, and Paraud, 2012; Wang *et al*., 2010; Urquhart *et al*., 1996).

The mean oocysts count from infected animals was 2390 OPG (range 300 to 12,500). The mean was meaningfully higher ($p< 0.05$) in lamb and young, female, and poor body condition sheep. In addition, it was statistically significant ($p< 0.05$) in sheep with soft faecal consistency and semi-intensively managed (Table 2). Higher oocyst counts were reported in lamb and/or young sheep (Chartier and Paraud, 2012; Arslan *et al*., 1999; Maingi and Munyua, 1994; Kanyari, 1993) and the semi-intensive production systems (Chartier and Paraud, 2012) from various areas. The negative association between OPG and the age of sheep was a result of acquired immunity (Kanyari, 1993). Factors inducing stress, for example, increased number of animals in an area like in the semi-intensive production system, and loss of body condition might increase the oocysts output.

In a nut shell, the present study revealed that the prevalence of *Eimeria* species infection in sheep is higher. And it was significantly associated with various host-related (i.e. Age, sex, body condition, and faecal consistency) and environmental factors (i.e. Production system). So, this result provided useful information to design and implement appropriate control strategies. Finally, it is recommended that further to study identify the species of *Eimeria* circulating in the areas.

Limitation of the study

The major limitation of the study was lack of facilities for Eimeria spp. identification.

List of Abbreviations

CSA: Central Statistical Authority; OPG: Oocysts per gram of faeces; ROC: Receiver operating characteristic curve.

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