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Gastrointestinal nematodiasis in Ethiopian sheep under community-based breeding program

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Gastrointestinal nematodes (GIN) are amongst the main health and production problems in sheep throughout the world. Faecal egg count (FEC) and FAffa MAlan CHArt (FAMACHA) score can be used as indictors to select genetically resistant animals against the infections. This study was undertaken to (1) determine intensity of and the factors associated with GIN infections in Ethiopian sheep under communal breeding program, and (2) examine relationships between the intensity of GIN infection and levels of anaemia related to blood feeding GIN parasites. A total of 1239 FEC and FAMACHA scores were measured on two Ethiopian sheep breeds during rainy and dry seasons. The data were analyzed using the mixed model procedure, accounting for differences in fixed effects of breed, season and their interaction and a random effect of animal. The interaction of breed and season (p < 0.01) influenced the intensity of infections with GIN. There was no significant (p > 0.05) relationship between FEC and FAMACHA scores; hence the latter is not a suitable indicator of infections with GIN in these animals. FEC should be recorded rather than FAMACHA as a nematode resistance trait to be incorporated into the sheep breeding programs of Bonga and Horro, Ethiopia.

Key words: Breeding program, Ethiopia, faecal egg count, FAffa MAlan CHArt (FAMACHA), gastrointestinal nematodes, sheep.

INTRODUCTION

Sheep is an economically important livestock species in Ethiopia (Leta and Mesele, 2014), and is ranked second to cattle by population (Gizaw et al., 2013). However, due to constraints emanating mainly from inadequate genetic and health improvement programs, sheep production and productivity remain low. Several attempts to sheep breeding programs in the country, mainly with a crossbreeding strategy have failed (Duguma, 2010), in part because of lack of participation of sheep farming communities in the breeding programs. Cognizant of this,

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community-based breeding programs (CBBP) for three local sheep breeds of the country (Bonga, Horro and Menz) have been designed and implemented since 2009 (Duguma, 2010; Mirkena et al., 2012). Gastrointestinal nematodes (GIN) of sheep are worldwide problems of health, production and welfare (Roeber et al., 2013; Mavrot et al., 2015; Traoré et al., 2017). Similar problems may appear also in CBBP. Prior to designing appropriate GIN control strategy for CBBP, possibly with selective breeding, a better understanding of intensity and associated factors is needed. Factors determining the prevalence and severity of infection with GIN in sheep include: host-related (age, immunity, sex); parasite-related (survival and development of larvae in the environment, nematode species and their location in the host), and environmental factors (climate, weather, season and microclimate) (Roeber et al., 2013).

One way of measuring infection levels of GIN is by quantifying the number of eggs being passed in the faeces. Relatively high and low fecal egg counts (FEC) are usually seen in young and adult animals, respectively (Miller and Horohov, 2006). Selection for low FEC can be used to genetically enhance resistance to GIN parasites in growing lambs (Notter et al. 2017a), thereby incorporation of recording FEC into the CBBP could be possible. Another method, the (FAffa MAlan CHArt (FAMACHA) system, can be used to identify the ability of the animal to cope with GIN infection; hence allowing animals for genetic selection and lowering of selection pressure on *Haemonchus contortus* for anthelmintic resistance (Wyk and Bath, 2002; Notter et al., 2017b). *H. contortus* is a haematophagus GIN parasite, which may cause severe/fatal anemia in grazing sheep (Moors and Gauly, 2009; Roeber et al., 2013). Compared to FEC, FAMACHA scores are less expensive to record, providing opportunity to replace FEC as phenotypes for selection in situations with moderate to high H. contortus prevalence (Heckendorn et al., 2017). When this species is a predominant GIN infection in sheep, higher FAMACHA scores are associated with higher FEC (Kaplan et al., 2004; Notter et al., 2017b). The objectives of this study were to investigate gastrointestinal nematodiasis in Ethiopian sheep breeds under communal breeding programs, and to examine relationship between intensity of GIN and levels of anaemia related to haematophagous species of GIN.

MATERIALS AND METHODS

Study area and animals

The study was conducted in communities currently implementing CBBP for two local sheep breeds of Ethiopia, Bonga and Horro (Figure 1). The CBBPs were designed considering the communities' sheep population, which share communal grazing and watering points as one large flock (or a breeding unit) that comprised ≥ 400 breeding ewes when the implementation was commenced in 2009 (Duguma, 2010; Mirkena et al., 2012). The CBBP communities (two per breed) are located neighboring to each other. For Bonga sheep, these are Boka and Shuta which are situated at 26 and 29 km East of Bonga town. The altitudes of the CBBP sites range between 2500-2600 m above sea level (m a.s.l.), typically classed under highland agro-ecological zone (AEZ) of the country. Bonga is an administrative town of Kaffa Zone in Southern Nations Nationalities and People's Regional State (SNNPR), located about 450 km from Addis Ababa. The area around Bonga has the mean annual temperature of 12 to 25°C and mean annual rainfall of 2300 mm. For Horro sheep, the CBBP communities are Gitlo and Laku which are situated at about 7 km from Shambu and 3 km apart from each other. The CBBP sites have altitude ranging between 2700-2800 m a.s.l. (highland AEZ). Shambu is an administrative town of Horro Guduru Welega Zone in Oromia Regional State, located about 315 km from Addis Ababa. The area around Shambu has the mean annual temperature of 12 to 23°C and mean annual rainfall of 1800 mm. The detailed characteristics of Bonga and Horro sheep breeds were described in the previous studies (Gizaw et al., 2007; Mirkena et al., 2012). In brief, both sheep are long fat-tailed breeds and highly valued for their meat production (Mirkena et al., 2012).

Sampling for FEC determination and FAMACHA scoring

A total of 1239 FEC and FAMACHA scores were sampled (Table 1) during two main seasons (rainy: July through September 2016; dry: December 2016 through February 2017) from Bonga CBBP (rainy, n=324; dry, n=235) and Horro CBBP (rainy, n=391 and dry, n=289). Animals of both sexes and all ages over 2 months in the CBBP were represented in the sample. The FEC was determined by McMaster egg counting technique, following procedures described by Urquhart et al. (1996). FAMACHA scoring was performed by classifying color of conjunctival mucous membranes of each sheep according to Kaplan et al. (2004) and Burke et al. (2007) into five categories: 1 = red, non-anemic; 2 = red-pink, non-anemic; 3 = pink, mildly anemic; 4 = pink-white, anemic; 5 = white, severely anemic.

Statistical analysis

FEC data were analyzed in SAS 9.4 (SAS Institute Inc 2012) using the mixed model procedure, after log transformation [Ln (FEC + 25)] to conform normality. The constant was added to include zero FECs. The model was fitted using the effect of animal identity as random to account for measurements on the same animal during two seasons. The fixed effects included were breed/location, season and interaction between the breed and the season, as follows:

$$y_{ijk} = \mu + breed_i + animal_{ij} + season_k + (breed * season)_{ik} + e_{ijk}$$

where y_{ijk} is the response variable of log transformed FEC, μ is overall mean, $breed_i$ is the fixed effect of the i^{th} breed (Bonga or Horro); $animal_{ij}$ is the random effect of animal j within breed i; $season_k$ is the fixed effect of the k^{th} season (rainy or dry); $(breed * season)_{ik}$ is the interaction of breed by season; e_{ijk} is the random error. The FEC results are presented as both log transformed and back-transformed least squares means and standard errors (LSM ± SE). Non-transformed FAMACHA scores were analyzed in the same way. Relationship between FEC and FAMACHA scores was explored by boxplots and further examined using Kruskal-Wallis Test. For this purpose, differences in FEC between classes of FAMACHA were tested for each pair of FAMACHA scores. The boxplots were constructed using R (R Core Team, 2017). Spearman's correlation of FEC and FAMACHA was also computed.

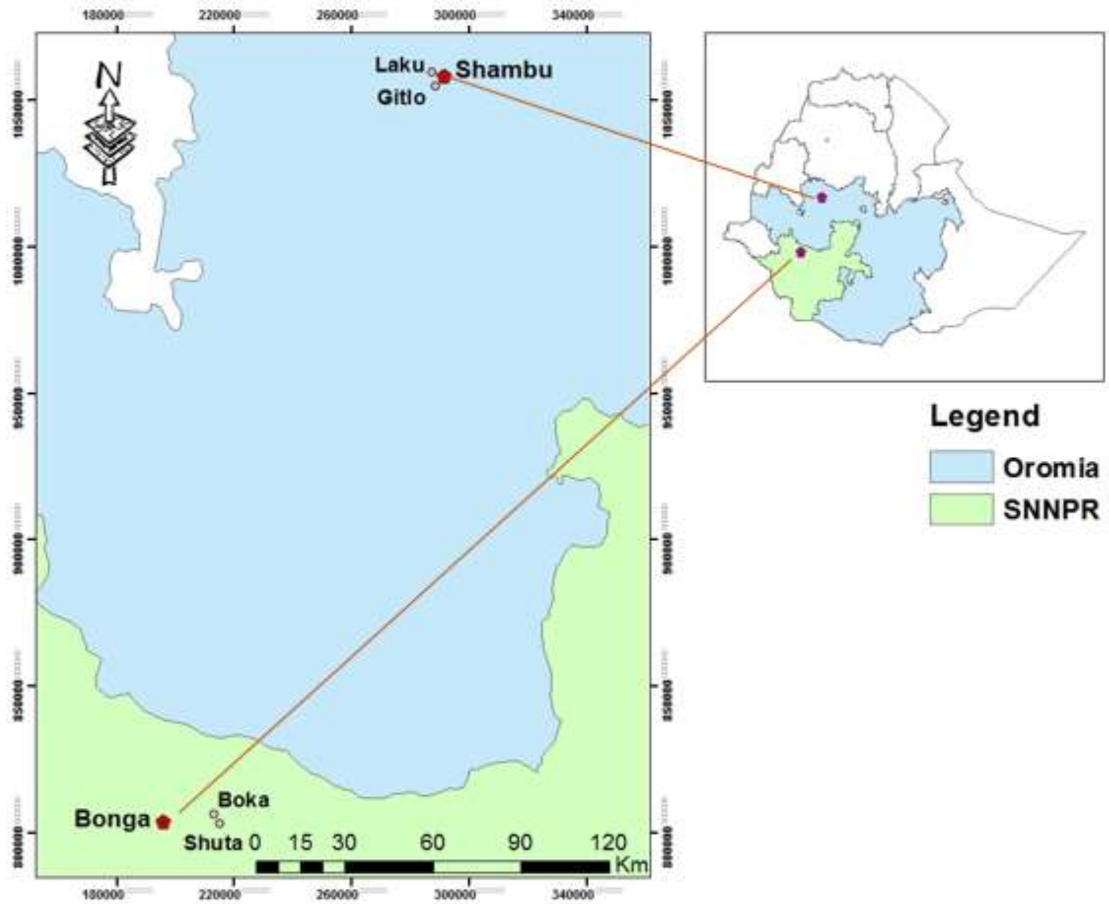

Figure 1. Map of Ethiopia illustrating the study sites for Horro sheep (Laku and Gitlo, in the vicinity of Shambu town, Oromia Region) and Bonga sheep (Boka and Shuta, nearby Bonga town, Southern Nations Nationalities and People's Regional State (SNNPR)).

Table 1. Number of sampled sheep for FEC and FAMACHA scores during two seasons from community-based breeding programs in Bonga and Horro, Ethiopia.

CBBP	Season						Total FEC and FAMACHA
	Rainy			Dry			
	FEC	FAMACHA	FEC and FAMACHA	FEC	FAMACHA	FEC and FAMACHA	
Bonga	388	324	324	310	236	235	559
Horro	530	391	391	398	378	289	680
Total			715			524	1239

RESULTS

Effects of breed and season on *FEC*

Table 2 shows factors associated with FEC under communal sheep breeding in Ethiopia. Season, breed and the interactions thereof, significantly ($p < 0.05$) influenced FEC. The least square means (LSM) and standard errors (SE) of log transformed FEC (or back-transformed FEC) during the dry season were 5.78 ± 0.08 (298.87 ± 10.72) and 3.82 ± 0.07 (20.727 ± 1.48), respectively, in Bonga and Horro CBBP. The corresponding values during the rainy season were 5.05 ± 0.07 (131.11 ± 9.38) and 4.91 ± 0.06 (110.02 ± 6.73).

Effects of breed and season on FAMACHA

Least square means (LSM) ± standard errors (SE) of FAMACHA scores for the effects of breed and season in

Table 2. Least square means (LSM) ± standard errors (SE) of log transformed FEC and back-transformed FEC and FAMACHA scores for the effects of season, breed and their interactions in sheep under CBBP.

Effect	LSM ± SE				
	N	Log transformed FEC	Back-transformed FEC	N	FAMACHA score
Season		*			ns
Dry	701	4.80 ± 0.05	96.69 ± 5.18	614	2.56± 0.04
Rainy	918	4.98 ± 0.05	120.18 ± 5.66	715	2.56 ± 0.03
Breed/Location		**			***
Bonga	698	5.42 ± 0.05	199.85 ± 10.72	560	2.48 ± 0.04
Horro	921	4.36 ± 0.05	53.57 ± 2.52	769	2.64 ± 0.03
Breed × Season		***			**
Bonga dry	310	5.78[a] ± 0.08	298.87 ± 23.92	236	2.56[b] ± 0.06
Horro dry	391	3.82[c] ± 0.07	20.72 ± 1.48	378	2.57[b] ± 0.05
Bonga rainy	388	5.05[b] ± 0.07	131.11 ± 9.38	324	2.40[b] ± 0.05
Horro rainy	530	4.91[b] ± 0.06	110.02 ± 6.73	391	2.72[a] ± 0.05

[a,b,c]LSM with different letters within the same column and effect are statistically different (p<0.05). Significance of effects: ns = not significant; *= significant at p < 0.05; ** = significant at p < 0.01; *** = significant at p<0.001.

sheep under CBBP are presented in Table 2. Breed/location affected FAMACHA score ($p < 0.001$), while season had no effect ($p > 0.05$) on the trait. A significant ($p < 0.01$) interaction was found between breed and season in FAMACHA.

Relationship between intensity of GIN infection and levels of anaemia

The relationship between intensity of GIN infection and levels of anaemia is shown by boxplots (Figure 2a to d) for Bonga and Horro sheep during rainy and dry seasons. There were no significant differences in FEC between FAMACHA classes: Kruskal-Wallis Test; $\chi^2 = 1.53\text{-}4.643$, df = 4, $p > 0.05$) in both breeds and seasons, though FAMACHA score increased with mean FEC in Bonga CBBP during rainy season. Also, Spearman's correlation test showed no evidence of significant relationships ($p > 0.05$) between the FEC and FAMACHA scores in both breeds and seasons (Table 3).

DISCUSSION

The pattern of interaction between season and breed showed that Bonga sheep had higher values of FEC than Horro sheep in the dry season. This could be attributed to the differences in agroclimatic factors of the breeds' locations. Our findings of a higher FEC during rainy season compared to the dry season in Horro sheep are in line with most studies in Ethiopia (Haile et al., 2010; Aga et al., 2013) and elsewhere in the world (Nwosu et al., 2007; Khan et al., 2010; Khajuria et al., 2013). But a lower FEC during the rainy season than the dry season in Bonga was inconsistent with these reports. This is possibly due to sheep flock management practices by farmers in this region; tethering of sheep on private land during the rainy season in Bonga may have lowered pasture contamination with nematode larvae, thereby reduced subsequent infection. This may alternatively be explained by the grazing of sheep on communal land (usually mixed with other livestock) coupled with a better precipitation received during the dry season in Bonga might have increased the risk of GIN infection. Though unexpected during the dry season, Abebe et al. (2010) also reported a high rate of GIN infection in sheep and goats, even a higher infection rate than our finding in the same region of southern Ethiopia. Generally, the FECs indicate that the intensity of infections with GIN was low in the CBBP of Bonga and Horro during both rainy and dry seasons. Taylor (2010) suggested classification of intensity of GIN infection in sheep based on FEC values: FEC < 500 as low infection; FEC = 500-1000 as moderate infection; FEC more than 1000 as high infection.

The least square means and standard errors of FAMACHA scores suggested that the levels of anaemia were mild. The differences of the least square means revealed that the FAMACHA scores in Horro sheep during rainy season were significantly different from the other groups. However, this highest level of anaemia may not be due to haemonchosis as indicted by absence of significant correlation between FEC and FAMACHA. Also, the correlation was negative (though non-significant), indicating that the cause of anaemia could be other factors rather than *H. contortus*. FAMACHA method suffers from the problem of non-specificity, for example, anaemia in sheep can be caused by many factors, consideration of other haematophagous parasites is always necessary, particularly Fasciola (Wyk and Bath, 2002; Kaplan et al., 2004).

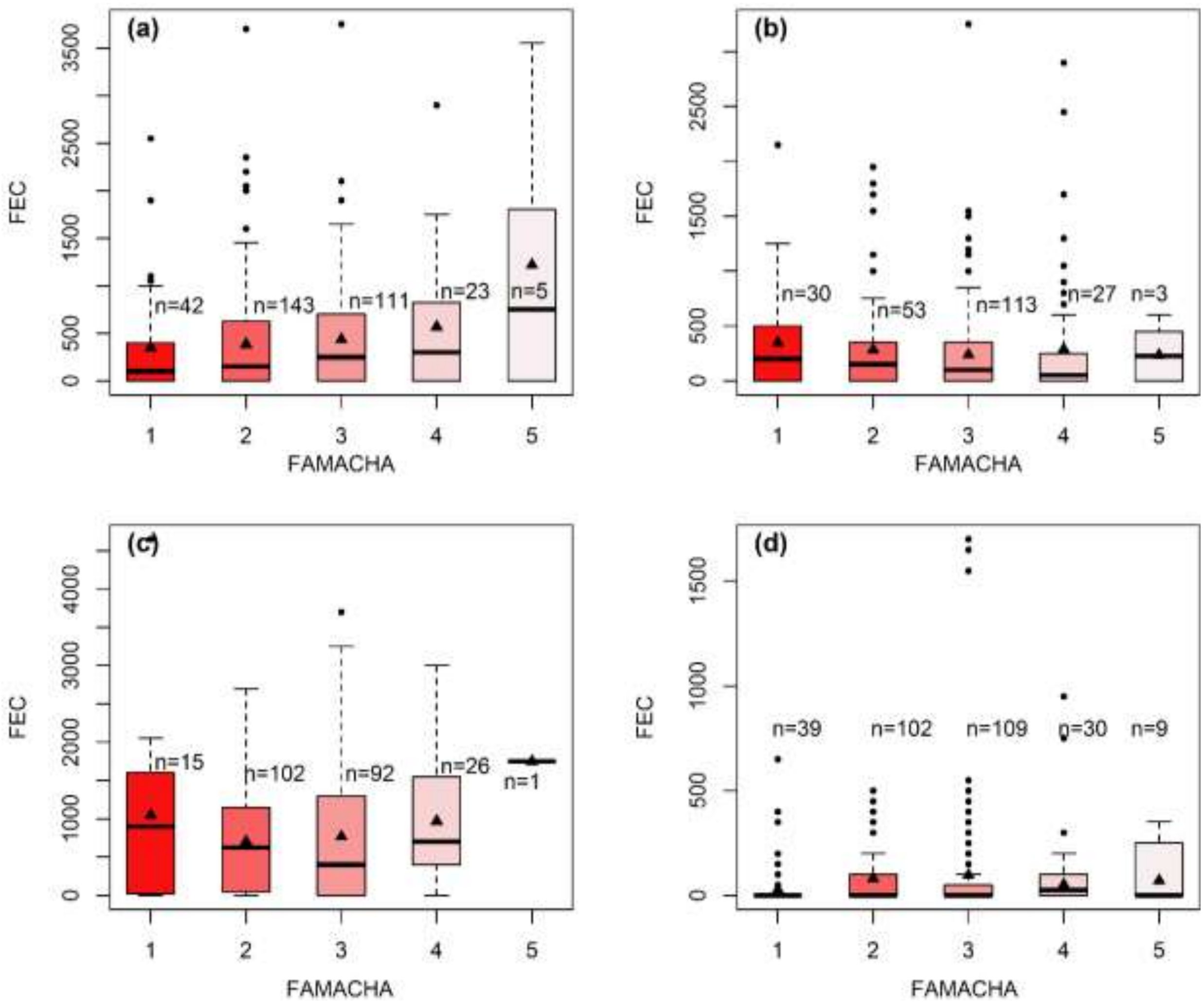

Figure 2. Boxplots showing the relationship between FEC and FAMACHA scores in CBBP sheep: (a) Bonga, rainy season; (b) Horro, rainy season; (c) Bonga, dry season; (d) Horro, dry season. Solid triangles show the mean and solid lines show the median of FEC in each FAMACHA category. The lower and upper boundaries of the boxes reveal the 1st and 3rd quartiles. The whiskers below and above the boxes indicate minimum and maximum non-outliers, and the circles (filled black) show outliers, and "n" indicates the number of observations.

Table 3. Spearman's correlation (r) between FEC and FAMACHA score in Bonga and Horro sheep in CBBP during rainy and dry seasons.

FAMACHA	FEC					
	Bonga			Horro		
	N	r	p value	N	r	p value
Rainy	324	0.05	0.409	235	-0.09	0.094
Dry	391	0.04	0.576	289	0.11	0.069

There was a tendency for the FAMACHA score to increase with the mean FEC in Bonga sheep during rainy season, though the correlation between them was not significant. The lack of association indicates that H.contortus is not a highly dominant GIN parasite in sheep of Bonga and Horro CBBP. This low prevalence of the parasite might be due to the higher altitudes of the present CBBP locations (≈ 2500 m). In lower altitudes (< 1500 m), however, H.contortus has been reported to be a dominant GIN species and may cause life threatening disease in all age groups of sheep (Balmer et al. 2015). Our finding is supported by Aga et al. (2013), who based on coproculture identification of nematode species, reported a lower prevalence rate of *H. contortus* in highland (16.1%) than midland and lowland (37.5 and 40.0%) in Horro sheep in Western Oromiya, Ethiopia. The applicability of the FAMACHA method is limited when a percentage of *H. contortus* in the flock is not greater than 60% (Vilela et al., 2012). A slightly lower prevalence of *H. contortus* (58.9%) in goat flocks under field conditions in Switzerland, Scheuerle et al. (2010) reported a significant correlation between FAMACHA and FEC in only one out of six occasions. Similarly, with a very lower prevalence of *H. contortus* (12-34%) in German sheep, Moors and Gauly (2009) did not find any significant correlation between FAMACHA and FEC. Other similar field studies conducted in Northern Germany on naturally infected sheep and goats by Koopmann et al. (2006) showed that at a comparatively low prevalence of H. contortus, the FAMACHA system proved not being sufficient in detecting all animals with high FEC.

Conclusion

In conclusion, the intensity of infection with GIN in sheep of Bonga and Horro under CBBP were low to moderate. FAMACHA was found not to be a suitable indicator of GIN infections in CBBP of Bonga and Horro. Therefore, FEC should preferably be recorded and incorporated as a nematode resistance trait into the CBBP. However, routine recording of FEC under the CBBP condition may be difficult due to complex logistics and trained personnel required to do the laboratory work.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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