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# Control practices of gastrointestinal nematodes and inbreeding of Ethiopian sheep managed in community-based breeding programs

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## Abbreviations and Acronyms

| | |
|---|---|
| AEZ | Agroecological zone |
| CBBP | Community based breeding program |
| FAMACHA | FAfa MAlan CHArt |
| FEC | Faecal Egg Count |
| $F_{ROH}$ | Genomic inbreeding coefficient |
| GIN | Gastrointestinal nematodes |
| IBD | Identity by descent |
| ICARDA | International Center for Agricultural Research in the Dry Areas |
| ILRI | International Livestock Research Institute |
| m a.s.l | Meters above sea level |
| PE | Participatory epidemiology |
| ROH | Runs of homozygosity |
| SAS | Statistical Analysis System |
| SNP | Single Nucleotide Polymorphism |
| Spp | Species |
| SPSS | Statistical Package for the Social Sciences |

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## Abstract

Control of gastrointestinal nematodes (GIN) and inbreeding are important for improving health and productivity of sheep. The objectives of this thesis were to determine the intensity of GIN infections and its relationship with level of anaemia; assess the indigenous knowledge, practices and control options of GIN; and analyze genomic inbreeding and fine-scale structure of Ethiopian sheep managed under community-based breeding programs (CBBP).

A total of 1239 faecal egg count (FEC) and visual inspections of the mucosa for anemia (with FAMACHA scores) were measured on Bonga and Horro sheep during rainy and dry seasons to determine the intensity of GIN infections and its relationship with level of anaemia. A questionnaire survey (n = 240) in combination with participatory epidemiology were used to assess the indigenous knowledge, practices and control options of GIN. High density SNP genotype data were used for analysis of inbreeding and population fine structure.

The intensity of infections with GIN was low in the CBBP. No relationship was observed between FEC and FAMACHA scores in both breeds and seasons, indicating that FAMACHA may not be used as GIN indicator in the regions involved. Diarrhea and bottle jaw were among the most important disease conditions of sheep in CBBP. Ethnoveterinary medicinal plants are widely used in Bonga while the knowledge about them is at risk of loss in Horro. Anthelmintic use was significantly higher ($p < 0.001$) in CBBP than the non CBBP communities. In Horro, CBBP farmers considered anthelmintics as more sustainable GIN control option than non CBBP farmers ($p < 0.001$).

The average genomic inbreeding levels, considering ancestry as far as 50 generations back were 0.06 to 0.07 in three Bonga CBBP villages. High levels of inbreeding, pointing to parent-offspring or full sib mating, were extremely rare and levels of inbreeding indicating half sib mating were also rare. No evidence of structuring of Bonga sheep into subpopulations of CBBP villages was found.

In conclusion, there are low levels of GIN infection, high frequency of anthelmintics use and low levels of inbreeding in Bonga and Horro sheep managed under communal breeding schemes. It is suggested to reduce the use of anthelmintics and to include faecal egg count as additional trait in the breeding program, in order to reduce GIN by genetic means rather than provoking anthelmintic resistance.

**Keywords:** Ethiopia, sheep, gastrointestinal nematodes control, local knowledge, participatory epidemiology, genomic, inbreeding, population structure

## Zusammenfassung

## Lokale Praktiken der Kontrolle gastrointestinaler Nematoden und der Inzucht äthiopischer Schafe in dörflichen Zuchtprogrammen

Die Kontrolle gastrointestinaler Nematoden (GIN) und von Inzucht sind wichtig, um die Gesundheit und Produktivität von Schafen zu verbessern. Die Ziele dieser Arbeit waren, die Intensität der GIN-Infektion und deren Beziehung zur Anämie zu prüfen; das lokale Wissen und die angewandten Methoden der Kontrolle von GIN zu untersuchen; sowie die genomische Feinstruktur und die Inzuchtgrade von äthiopischen Schafen zu ermitteln, welche im Rahmen von dörflichen Zuchtprogrammen (community based breeding programs – CBBP) gehalten wurden.

In den Regionen Bonga und Horro wurde Anzahl von Nematoden-Eiern (fecal egg count –FEC) aus 1239 Kotproben ermittelt und ein Check von Anämie durch visuelle Inspektion der Mucosa des Auges mit der sogenannte FAMACHA Methode wurde bei den Tieren durchgeführt, deren Kot gesammelt wurde. Damit sollte die Intensität der GIN-Infektionen und deren Zusammenhang mit Anämie ermittelt werden. Mit einer Analyse von 240 Fragebögen-und von den Ergebnissen von Workshops zur partizipativen Epidemiologie wurde lokales Wissen zu GIN sowie zu Methoden deren Kontrolle untersucht. Mit genomischen Analysen von Hochdurchsatz single nucleotide polymonphism (SNP) Genotypen von insgesamt 175 Tieren wurden Inzuchtgrad und Populations-Feinstruktur von Tieren aus CBBP in Bonga untersucht.

Zwischen FEC und FAMACHA konnte in der vorliegenden Studie kein signifikanter Zusammenhang ermittelt werden. FAMACHA kann deshalb in der Region nicht als Indikator für GIN-Belastung verwendet werden. Durchfall und Kehlgangsödem waren die häufigsten Krankheitssymptome . Ethnoveterinäre Pflanzen wurden in Bonga häufig verwendet, während das Wissen darüber in Horro großteils verloren ging. Entwurmungsmittel wurden in CBBP

sehr häufig verwendet, signifikant häufiger als in Dörfern, in denen kein solches Zuchtprogramm implementiert wurde.

Der durchschnittliche genomische Inzuchtkoeffizient für eine Ahnenreihe von 50 Generationen lag in drei CBBP-Dörfern in Bonga zwischen 0.06 und 0.07, starke Inzucht durch Elter-Nachkommen- oder Vollgeschwister-Paarung war extrem selten. Analyses zur Feinstruktur der dörflichen Populationen zeigten keinerlei genetische Substruktur. Zusammenfassend kann festgestellt werden, dass in den untersuchten Dörfern mit CBBP in Bonga und Horro die Frequenz von GIN niedrig, der Einsatz von Entwurmungsmitteln hoch sowie der Inzuchtgrad der untersuchten Schafe niedrig war. Es wird empfohlen, den Einsatz von Entwurmungsmitteln zu reduzieren und die Selektion gegen FEC als züchterisches Mittel in den untersuchten CBBP Dörfern zu implementieren.

**Schlüsselworte:** Äthiopien, Schaf, Zuchtprogramm, Gastrointestinale Nematoden, Inzucht, Populationsstruktur, Lokales Wissen, Partizipative Epidemiologie

# 1 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 (Gizaw et al., 2013; Biffa et al., 2006). 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, 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). According to Duguma (2010) the CBBP project included seasonal mass de-worming of animals, considering that the health intervention increases productivity in the short-term, and thereby assure farmers' motivation well before the first positive breeding effects become visible. However, this increases risk of anthelmintic resistance, necessitating an alternative control method for nematode parasites. From the sustainability perspective, nematode resistance ought to be incorporated into the breeding goal traits of the CBBP.

Gastrointestinal nematodes (GIN) of sheep are worldwide problems of health, production and welfare (Mavrot et al., 2015; Roeber et al., 2013; Traoré et al., 2017). Similar problems may appear also in CBBP. 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).

Several methods have been advocated to control GIN, including anthelmintics, ethnoveterinary medicine, grazing management, nutritional supplementation, genetic approaches, biological methods and vaccination (Githiori et al., 2006; Athanasiadou et al., 2007; Stear et al., 2007). The utilization of these control options depends on their availability, effectiveness, cost, ease of implementation and sustainability (Stear et al., 2007; Stear, 2010). Anthelmintic treatment offers a simple, cheap, effective, and readily available method of nematode control (Stear et al., 2007). However, anthelmintics may not be always available to all livestock farmers. When available their use is hampered due to concerns with anthelmintic resistance, animal welfare issues and public health concerns (Getachew et al., 2007). Hence, the use of anthelmintics in the control of nematodes is not sustainable (Stear, 2010). Existing methods for nematode control that can be used in place of anthelmintics have their own advantages and disadvantages. Despite ethnoveterinary medicinal plants have been used for long by farmers and traditional healers to treat parasitism, scientific evidence on the antiparasitic efficacy of most plant products is limited (Githiori et al., 2006). Genetic approaches include the use of resistant breeds and selective breeding of individuals resistant to GIN. Selective breeding is effective and inexpensive but requires a high level of expertise (Stear et al., 2007). 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 FAMACHA scoring system, which classify color of conjunctival mucous membranes of animals from 1-5, indicating normal to anaemic (Burke et al., 2007), 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 (Notter et al., 2017b; Wyk and Bath, 2002). *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 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., 2017).

Mating between individuals related by common ancestry results in inbreeding, which in turn leads to a decline in performance, inbreeding depression (Blouin and Blouin, 1988; Curik et al., 2014; Ferenčaković et al., 2013). Inbreeding is inevitable in populations under selection as only a subset of individuals is used for breeding (Marras et al., 2015); hence, it is an important parameter to monitor and control in breeding programs (Grilz-Seger et al., 2018; Norberg and Sørensen, 2007), such as in CBBP. Inbreeding has traditionally been estimated from pedigree information (Marras et al., 2015). In absence or incompleteness of pedigrees (Grilz-Seger et al., 2018; Purfield et al., 2012), as is often the case in developing countries, it can be accurately derived from runs of homozygosity (ROH) using molecular information, notably single nucleotide polymorphisms (SNPs) (Keller et al., 2011; Peripolli et al., 2018, 2017). The ROH are contiguous segments of homozygous genotypes that are present in an individual due to parents transmitting identical haplotypes to their offspring (Purfield et al., 2012). Lengths of ROH provide information on inbreeding history: longer haplotypes are typically inherited from recent common ancestors and shorter haplotypes from distant ones (Ceballos et al., 2018). Advances in high-throughput SNP genotyping technologies along with reducing cost for genotyping (Kijas et al., 2012) provides the opportunity to use the SNP data in developing countries, among others, for analyzing diversity, population structure and, inbreeding of sheep populations. Assessing population fine structure of sheep in CBBP may reveal aspects of breeding history of the sheep among the CBBP villages and whether CBBP have created a substructure in the sheep populations.

Prior to designing appropriate GIN control strategy for CBBP, possibly with selective breeding, a better understanding of intensity and associated factors, local knowledge, current practices and preferences for different control options is needed. Likewise, monitoring and controlling of inbreeding in CBBP is crucial. Therefore, the objectives of this thesis were to: 1) determine the intensity of GIN infections and its relationship with level of anaemia in Ethiopian sheep managed under communal breeding scheme; 2) assesses the indigenous knowledge, practices and control options of GIN in Ethiopian sheep managed under communal breeding scheme; and 3) analyze genomic inbreeding and fine-scale structure in Ethiopian sheep managed under communal breeding scheme.

# 2 Literature Review

## 2. 1 Sheep production in Ethiopia

### 2. 1.1 Population size and roles

Sheep are the second most important species of livestock in Ethiopia (Gizaw et al., 2013). The population size of Ethiopian sheep is estimated to 29.3 million heads (CSA, 2015). They have multi-purpose roles in the country, among others, for source of income (from sale of live animals), food (meat and milk) and manure (Edea, 2008; Leta and Mesele, 2014).

### 2.1.2 Breeds

Fourteen traditional sheep populations of Ethiopia, characterized based on morphology, community and geographic distribution, were classified into six breed groups and nine breeds using microsatellite markers (Gizaw et al., 2007). Bonga and Horro are among those distinct breeds. Bonga sheep have brown or brown with white color and are fat-tailed (Mirkena et al., 2012; Edea, 2008). Horro sheep have predominantly brown or creamy white color (Edea, 2008), short smooth hair, a triangular fat tail with relatively narrow base and with the pointed end hanging downward or with a slight twist (Galal, 1983).

### 2.1.3 Production systems

Major production systems of sheep in Ethiopia are mixed crop–livestock system, pastoral and agropastoral production systems. Other production systems that are not currently practiced widely but have a future are ranching, urban and peri-urban (landless) sheep production systems (Abegaz et al., 2008). Smallholder livestock production predominates in the highland mixed crop–livestock systems because of land and capital limitations (Gizaw, 2010).

### 2.1.4 Constraints

Complex sets of interrelated factors which influence sheep production and productivity in Ethiopia include; feed shortage, poor infrastructure, lack of market information and technical capacity, absence of planned breeding programs and breeding policies, lack of involvement of farmers in designing and implementation of breeding programs, diseases and (helminth) parasites (Gizaw et al., 2013; Duguma, 2010; Biffa et al., 2006). Recently, efforts have been made to address some of the constraints, for instance, by participating farmers in development of communal sheep breeding programs in Ethiopia (Mirkena et al., 2012).

#### 2.1.5 Community based breeding programs (CBBP)

As described by Mueller et al. (2015), CBBP are breeding programs typically related to low-input livestock production systems in developing countries with farmers within limited geographical boundaries having a common interest to improve and share their genetic resources.

In Ethiopia, CBBP were developed for three local sheep breeds, namely Bonga, Horro and Menz (Duguma, 2010), as a response to failures of and/or alternative to top-down structured breeding programs like a crossbreeding (Mirkena et al., 2012). The project was initiated as a collaboration of the International Center for Agricultural Research in Dry Areas (ICARDA), the International Livestock Research Institute (ILRI) and the Austrian University of Natural Resources and Life Sciences Vienna (BOKU) and the Regional Agricultural Research Systems in Ethiopia (Duguma, 2010). When the implementation commenced in 2009, the CBBP were designed considering the communities' sheep population that share communal grazing and watering points as one large flock (or a breeding unit) comprising $\geq$ 400 breeding ewes (Mirkena et al., 2012; Duguma, 2010). Sheep farmers were involved in various activities during designing and implementation of the CBBP, such as definition of breeding goals and ram selection.

For the ease of implementation of CBBP for Ethiopian sheep, only few traits – up to three (production and reproductive) traits were considered (Mirkena et al., 2012). As the breeding programs progress, it is worth emphasizing on inclusion of disease resistance traits, such as nematode resistance into the breeding goal of the CBBP. Moreover, the CBBP project included seasonal mass de-worming of animals, considering that the health intervention increases productivity in the short-term, and thereby assures farmers' motivation well before the first positive breeding effects becoming visible (Duguma, 2010). However, this increases risk of anthelmintic resistance development in sheep nematodes, necessitating an alternative control method. From the sustainability perspective, therefore, nematode resistance ought to be incorporated into the breeding goal traits of the CBBP.

### 2.2 Gastrointestinal nematode (GIN) infections of sheep

GIN are parasitic nematodes (roundworms) which belong to the order Strongylida and reside in the gastrointestinal tract of sheep and other ruminants. They have been a major factor limiting sheep production worldwide (Roeber et al., 2013; Vlassoff et al., 2001), which can cause parasitic gastroenteritis typically in young animals and this disease provokes clinical signs, such as diarrhea, reduced growth and weight loss (Vande Velde et al., 2018).

#### 2.2.1 Main species

The principal species of GIN affecting sheep in different regions of the globe include *Haemonchus contortus*, *Teladorsagia* spp and *Trichostrongylus* spp (Besier et al., 2016). The prevalence of these GIN parasites can vary even within a country in the tropics. For instance, proportion of *H. contortus* was reported to be low in sheep in the highlands of Ethiopia (Aga et al., 2013; Rege et al. 2002) while it was the most prevalent parasite in sheep in the semi-arid region of eastern Ethiopia (Sissay et al., 2007). The main pathogenic effects of *H. contortus* are due to feeding on blood, causing severe anaemia. Acute disease is usually dependent on the intensity of infection, and is associated with signs of haemorrhagic anaemia, dark-coloured faeces, oedema, weakness, reduced production of wool and muscle mass, or sometimes sudden death. In cases of chronic disease, decreased food intake, weight loss and anaemia are most commonly observed (Roeber et al., 2013).

2.2.2 Life cycle and transmission

The basic life cycle of GIN of sheep is shown in Figure 1. Adult worms in the gastrointestinal tract mate and females lay eggs containing the developing embryos, which pass out in the sheep's faeces (Vlassoff et al., 2001). Within the faeces the eggs undergo further development through two feeding stages (first and second stages, respectively, $L_1$ and $L_2$,) to the non-feeding infective stage ($L_3$) that migrate onto the herbage in ensheathed form. Infection of the host occurs by ingestion of $L_3$. Following ingestion by a suitable host the $L_3$ larvae exsheath before they reach their site of infection. They undergo two further moults and complete their development (Roeber et al., 2013; Vlassoff et al., 2001).

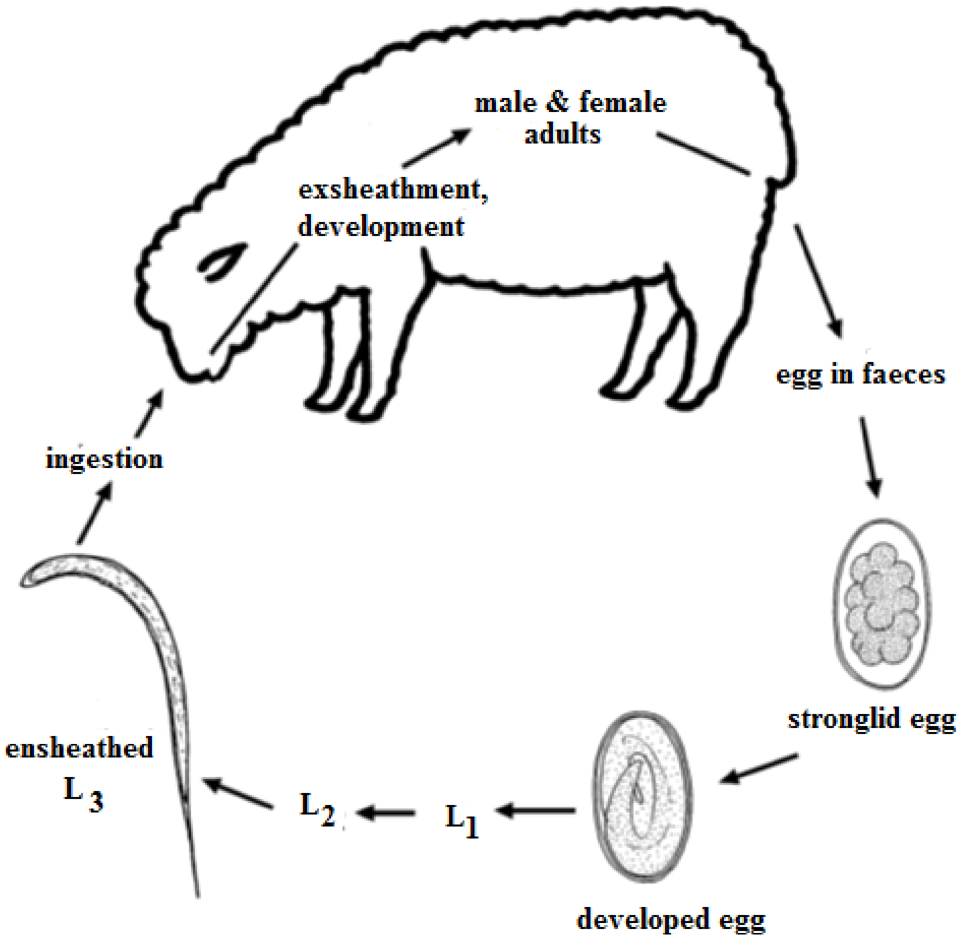



**Figure 1** Life cycle of gastrointestinal nematodes (GIN) of sheep. $L_1$, $L_2$, and $L_3$ represent the first, second and third stages of larvae (adapted from Roeber et al., 2013).

### 2.2.3 Epidemiology

Epidemiology is the study of the distribution of infections in animal populations and the characteristics of factors that are associated with these diseases (Tariq et al. 2015). The epidemiology of GIN depends on the aspects of relationship among host, parasite and environment and many factors linked to this relationship determine the prevalence and intensity of infection with the parasite. Host-related factors are age, immunity, sex, species and genetic resistance; parasite-related factors include life history, survival of larvae in the environment and their location in the host; environmental factors include climate, weather, season, type of vegetation and microclimate (Tariq et al. 2015; Roeber et al., 2013; Getachew et al., 2007). Knowledge of the epidemiology of GIN infections of sheep is important for planning, monitoring and assessment of GIN control programs (Thrustfield, 2005; Vlassoff et al., 2001). Several epidemiological studies of gastrointestinal nematode infections of sheep have been conducted in Ethiopia (e.g., Aga et al, 2013; Haile et al., 2010; Sisay et al., 2007), however this information is lacking for sheep managed in CBBP.

#### 2.2.3.1 Participatory epidemiology

Participatory epidemiology (PE) is an evolving branch of veterinary epidemiology which uses a combination of practitioner communication skills and participatory methods to improve the involvement of animal keepers in the analysis of animal disease problems, and the design, implementation and evaluation of disease control programs (Catley et al., 2012). It relies heavily on indigenous knowledge and terminology. The main methods for collecting information are by semi-structured interviews, scoring and ranking, and visualization (Thrusfield, 2005). In their review Catley et al. (2012) indicated the types of PE methods and veterinary information collected by the PE. These include simple ranking for analysis of disease control strategies; simple scoring for prioritization of livestock diseases; matrix ranking for analysis of disease control options and matrix scoring for local characterization of the clinical signs and causes of disease, etc. There have been many studies that used PE methods in developing countries, such as to investigate cattle trypanosomiasis in Kenya (Catley et al., 2002), cattle tick control methods practiced in Zimbabwe (Sungirai et al., 2016), medicinal plants used in the control of gastrointestinal parasites in donkeys in Ethiopia (Scantlebury et al., 2013), but very few have been reported on the use of the PE methods for gastrointestinal nematode infections of sheep. Lack of involvement of livestock producers in designing and implementation of livestock health development programs may lead to failure of the programs. Therefore, the use of PE methods would help in designing appropriate GIN control strategies for sheep in CBBP of Ethiopia.

#### 2.2.4 Diagnosis

Diagnosis of gastrointestinal parasitism is generally based on clinical signs, seasonal occurrence of disease, laboratory examination of faeces and, where possible, supported by postmortem examination. Most species of nematodes affecting the digestive tract cause diarrhea (Taylor, 2010). The clinical diagnosis of haemonchosis is based mostly on the detection of anaemia in association with a characteristic epidemiological picture and confirmed at postmortem by the finding of large numbers of *H. contortus* in the abomasum. The detection of impending haemonchosis relies chiefly on periodic monitoring for anaemia, including the FAMACHA conjunctival-colour index, or faecal worm egg counts (FEC) and other laboratory procedures (Besier et al., 2016). The FAMACHA system was developed in South Africa and it refers to 'FAfa MAlan CHArt' (Malan et al., 2001).

#### 2.2.5 Control

Efficient and welfare-friendly livestock production requires effective control of infections with nematodes (Stear et al., 2007). Methods for control of GIN in sheep and other ruminants include anthelmintics, ethnoveterinary remedies, nutritional supplementation, genetic approaches, grazing management, biological control, and vaccination. Use of these methods depend upon such factors as effectiveness, availability to local farmers, ease of implementation, cost effectiveness and sustainability (Stear, 2010; Athanasiadou et al., 2007; Getachew et al., 2007; Stear et al., 2007; Githiori et al., 2006).

*Anthelmintics*: These are broad-spectrum chemicals, predominantly belonging to three main classes: the benzimidazoles (albendazole, etc.), the macrocyclic lactones (ivermectin, etc.) and the imidazothiazoles (levamisole, etc.) (Roeber et al., 2013; Vande Velde et al., 2018). The use of anthelmintic drugs has been the mainstay of nematode control in sheep and other livestock since anthelmintic treatments offer simple, cheap, effective, and readily available method of nematode control (Steer, 2010). However, excessive and frequent use of the anthelmintics have led to widespread emergence of anthelmintic resistant strains of GIN parasites. Therefore, there is an increasingly urgent need to develop alternative or supplementary methods of nematode control for sheep in particular and other livestock in general (Roeber et al., 2013; Getachew et al., 2007; Stear et al., 2007).

*Ethnoveterinary remedies:* The use of herbal remedies for control of nematode parasites of livestock based on whole plants or their parts (leave, roots, flowers, blubs, etc.),  may offer a cheaper and an easily available alternative to commercial anthelmintics (Githiori et al., 2005), though scientific evidence on the antiparasitic efficacy of most plant products is limited (Githiori et al., 2006). Methods currently used to validate anthelmintic properties of medicinal plants are *in vitro* and *in vivo* studies. Using such evaluation, Iqbal et al. (2006) reported that *Nicotiana tabacum* was found to be effective in treatment of GIN of sheep in Pakistan, while Githiori (2004) concluded that the plant species evaluated (such as *Albizia anthelmintica*) were found to be ineffective as anthelmintics against the parasites of sheep in Kenya. The use of medicinal plants for the prevention and treatment of gastrointestinal parasitism has its origin in ethnoveterinary medicine (Athanasiadou et al., 2007), such information being  undocumented, remains in the memory of elderly practitioners, usually passed orally from generation to generation (Awas and Demissew, 2009).

*Genetic approaches:* These include the use of resistant breeds and selective breeding of individuals resistant to GIN, due to considerable evidence for genetic variation to the GIN parasites that has been found both between and within sheep breeds (Stear et al., 2007). The main use of genetic resistance is the selective breeding of resistant sheep to nematodes (Stear, 2010), which relies on indicators, such as FEC and FAMACHA (Besier et al., 2016; Notter et al., 2017a). Heritabilities of FEC for sheep, ranged between 0.01 and 0.44 (Stear et al., 2007; Gauly and Erhardt, 2001). Estimates of heritability for FAMACHA in sheep ranged from 0.06 to 0.24 (Riley and Van Wyk, 2009). Other reports of anaemia scores for sheep with haemonchosis, show moderate to high heritabilities (0.3-0.4) (Besier et al., 2016; Thamsborg et al., 2010). In several studies, where *H. contortus* was the predominant species of GIN parasite of small ruminants, higher FAMACHA scores were associated with higher FEC (Notter et al., 2017b; Heckendorn et al., 2017; Kaplan et al., 2004). However in other studies, no correlations have been reported between the two indicator traits for nematodes infection (Heckendorn et al., 2017; Moors and Gauly, 2009; Koopmann et al., 2006). This could be due to factors, such as higher altitudes above 1500 m, where *H. contortus* is not a predominant nematode species (Balmer et al., 2015). Although genetic selection is sustainable and effective, it requires a high level of expertise (Stear et al., 2007), and can be expensive due to need for faecal collections and the expense of laboratory determination of FEC (Notter et al., 2017b). Use of FAMACHA scores as an alternative to FEC in selection programs would be feasible, effective and less expensive, especially for resource-poor communities in developing countries where labour is relatively cheap and most farmers own small number of animals (Heckendorn et al., 2017; Riley and Van Wyk, 2009).

*Nutritional supplementation:* This is strategic feed supplementation, usually protein supplementation, especially to the most susceptible groups of sheep (young, pregnant or lactating ewes) to nematode infection (Stear et al., 2007; Waller and Thamsborg, 2004). The use of protein supplementation has been shown to offset stresses imposed by lambing, resulting in dramatic reductions in worm burdens and FEC of lactating ewes, precluding the need for anthelmintic treatments (Vlassoff et al., 2001). Though nutritional supplementation can be effective, it is expensive and awkward for sheep reared at low density under extensive management schemes (Stear, 2010).

*Grazing management:* This involves schemes to reduce the number of infective nematodes (third stage larvae) available to infect grazing animals. Options include stocking density, alternating use of pasture, move to clean pasture and rotational grazing (Stear et al., 2007; Waller et al., 2006). The method can be helpful to control nematode infections, but it is often impractical and insufficient on its own (Stear, 2010).

*Others (biological control and vaccination):* Biological control is the use of natural enemies of nematodes to reduce pasture contamination and parasite populations, an example is administration of fungi that feed on nematodes (*Duddingtonia flagrans*). The method can be as effective as anthelmintic drug treatment, but currently this option is limited by the need to administer fungal spores every day (Stear, 2010; Waller et al., 2006). Vaccination against nematode parasites holds great promise but there are no vaccines currently available (Stear, 2010).

*Factors influencing adoption of sustainable GIN control:* Adoption or uptake of agricultural innovations (Meijer et al., 2015) in general, and the uptake of sustainable GIN control in particular, are not only explained by rational choices (like financial motives), but also by farmer specific behaviors, which mainly consist of socio-psychological factors (e.g., knowledge, attitude, subjective norms, risk perception) (Vande Velde et al., 2018). A survey of helminth control practices on sheep farms in Great Britain and Ireland revealed the current helminthic control strategies on the sheep farms and the technical barriers to the uptake of alternative and sustainable methods (Morgan et al., 2012). The authors reported that anthelmintic use was influenced by past experience and perceived reliability of the drugs, along with convenience of use and price. The same authors also reported that low awareness of the risk of anthelmintic resistance and positive attitude toward their current use of anthelmintics were accordingly identified as barriers for the adoption of sustainable practices (Vande Velde et al., 2018).

### 2.3 Other helminth parasites of sheep

Apart from GIN, trematodes (mainly *Fasciola* spp) are helminth parasites of sheep having health and production importance in some regions. In the tropics, occurrence of infections with *Fasciola* spp can be year-round, demanding 3 to 4 anthelmintic treatments. High rainfall and wet pasture are suitable environmental conditions for clinical fasciollosis (manifested by bottle jaw, anaemia etc.), production loss (weight loss) and possibly death (Torgerson and Claxton, 1999). Anaemia due to fasciollosis need to be differentiated from haemochosis (Kaplan et al., 2004). Other helminth parasitic infections seen in sheep such as adult tapeworms (*Moniezia* spp) are generally of lesser importance (Taylor, 2010).

### 2.4 Inbreeding and population structure

#### 2.4.1 Inbreeding and inbreeding effects

Inbreeding resulting from mating of individuals related by common ancestry (Blouin and Blouin, 1988), has shown to negatively affect birth weight, average daily gain, and litter size in sheep, consequently, it is an important parameter to monitor and control in breeding programs (Norberg and Sørensen, 2007).

### 2.4.2 Methods of estimation of inbreeding

The degree of individual inbreeding is measured using the inbreeding coefficient (*F*), defined as the probability that two randomly chosen alleles at a homologous locus within an individual are identical by descent (IBD) with respect to a base population in which all alleles are independent (Ceballos et al., 2018; Keller et al., 2011). Traditionally inbreeding has been determined based on pedigree information (Marras et al., 2015), however, over the last decade the interest in using genomic information has increased as a response to development in high-throughput single nucleotide polymorphisms (SNP) genotyping technology (Curik et al., 2014; Keller et al., 2011; McQuillan, et al., 2008). In the latter case, the genomic inbreeding coefficient ($F_{ROH}$) may be accurately derived from runs of homozygosity (ROH, Figure 2), which are contiguous segments of homozygous genotypes that are present in an individual due to parents transmitting identical haplotypes to their offspring (Purfield et al., 2012). The lengths of ROH provide information on inbreeding history: longer haplotypes are typically inherited from recent common ancestors (indicating more recent inbreeding) and shorter haplotypes from distant ones (indicating more ancient inbreeding) (Ceballos et al., 2018; Curik et al., 2014).

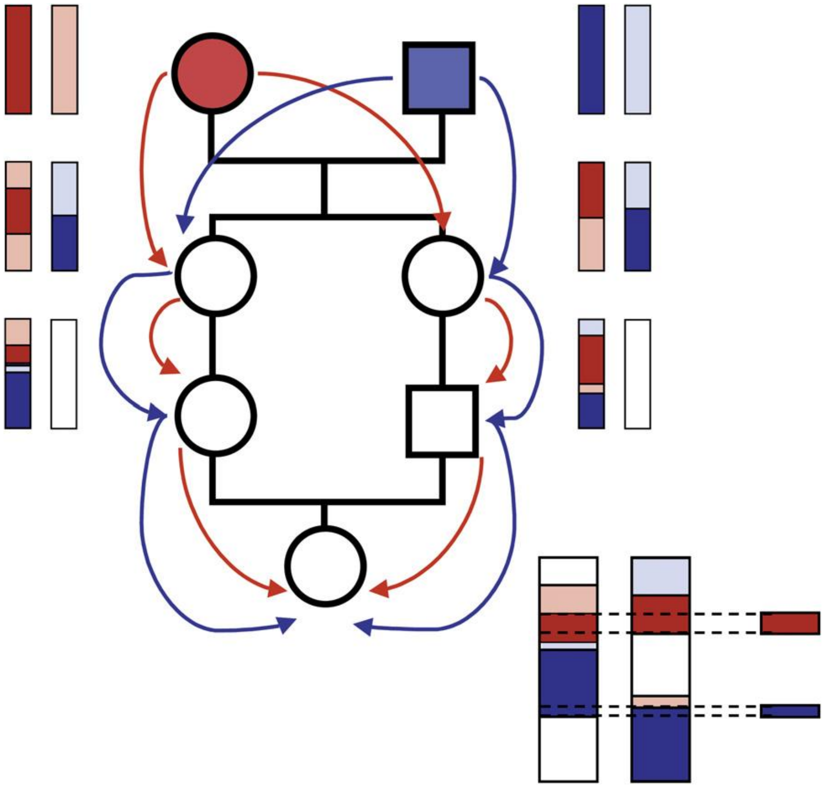


**Figure 2** A pedigree illustrating an offspring of first cousins which has segments inherited from both founders on both copies of the chromosome. Where the same segments have been passed down both sides of the pedigree, the offspring of first cousins has extended identical by descent (IBD) tracts or runs of homozygosity (ROH) (adapted from McQuillan, et al., 2008).

The major limitations of pedigree inbreeding coefficient ($F_{PED}$) are that it: (i) ignores stochastic differences in the proportion of the genome inherited IBD relative to the statistical expectation (Marras et al., 2015); (ii) fails to capture the influence of distant parental relationships, resulting in downward bias of inbreeding estimate (McQuillan, et al., 2008); (iii) fails to consider that in practice, pedigree information is difficult or impossible to collect or can be recorded inaccurately; and (iv) it assumes that the entire genome is selection neutral and does not account for potential bias resulting from selection (Peripolli et al., 2018; Keller et al., 2011).

Mueller et al. (2015) reported that recording of full pedigree information in village herds (CBBP) is difficult, which limits control of inbreeding. Thus, genomic estimates of inbreeding, using the $F_{ROH}$ method, can potentially be used in developing countries where pedigree recording is unavailable, or otherwise inaccurate, for management of inbreeding in sheep breeding programs, such as CBBP in Ethiopia.

### 2.4.3 Fine-scale population structure

Genetic clusters often corresponded closely to sets of geographically similar populations in human (Dobon et al., 2015; Rosenberg et al., 2002), cattle (McKay et al., 2008) and sheep (Gizaw et al., 2007; Kijas et al., 2009, 2012), among others. The most widely used markers to study genomic variation between and within populations of human and livestock are SNPs (Alhusain and Hafez, 2018; Kijas et al., 2009). The recent completion of genome sequence assembly of many species, including sheep, has provided sufficient numbers of SNP loci to replace microsatellite loci sequences for population genetic analyses (Kijas et al., 2009; McKay et al., 2008). The SNPs are markers of choice for such analyses due to their abundance in the genome, low mutation rate and low genotyping cost (Kijas et al., 2009; Kukučková et al., 2017).

The high-definition population networks by NetView can effectively visualize large- and fine-scale genetic structure within and between populations, including family-level structure and relationships (Steinig et al., 2016). Individuals can be effectively allocated to their correct population whilst simultaneously revealing fine-scale structure within the populations (Neuditschko et al., 2012). Identifying population fine structure of Ethiopian sheep in CBBP may reveal aspects of breeding history of the sheep among the CBBP villages as well as whether the CBBP have created substructures in the sheep populations. Based on the latter, rams use decisions across the CBBPs can be made.

# 3 Materials and methods

3.1 study area, community and animals

The study was conducted in two locations of Ethiopia: Bonga and Horro, targeting communities involved in CBBP (Figure 3).

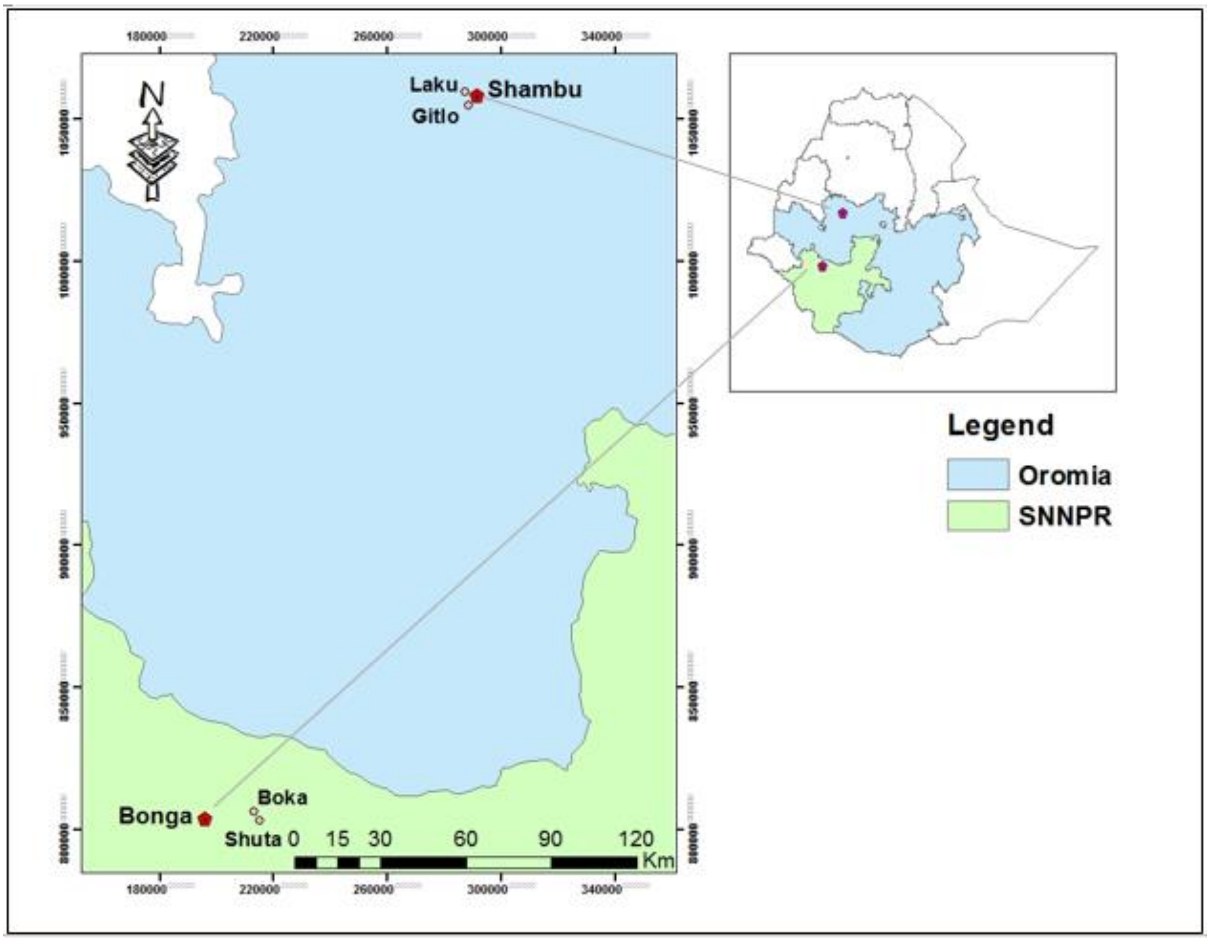



**Figure 3** Map of Ethiopia illustrating the study sites for Horro sheep (Laku and Gitlo, in the vicinity of Shambu town, Oromia Region) and Bonga sheep (Boka and Shuta, nearby Bonga town, Southern Nations Nationalities and Peoples Region (SNNPR)).

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 meter above sea level (m a.s.l.), typically classed under highland agro-ecological zone (AEZ) of the country. Bonga is the administrative town of Kaffa Zone, Southern Nations Nationalities and People's Regional State (SNNPR), located at about 450 km from Addis Ababa. The area around Bonga has a mean annual temperature of 12 to 25$^0$C and a 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 at a distance of 315 km from Addis Ababa. The area around Shambu has a mean annual temperature of 12 to 23$^0$C and mean annual rainfall of 1800 mm.

For the purpose of comparison, non CBBP communities found at a distance of 2 to 12 km from CBBP communities were considered for questionnaire survey. The non CBBP communities are similar to the CBBP communities in their respective area except that they are not involved in CBBP.

### 3.2 Data collection

#### 3.2.1 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.

**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 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Rainy | | | dry | | | Total FEC and FAMACHA |
| | 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 |

3.2.2 Questionnaire survey and participatory epidemiology data

A questionnaire survey in combination with participatory epidemiology (PE) methods was used to assess indigenous knowledge, practices and preferences in control of gastrointestinal nematodes in Bonga and Horro sheep of Ethiopia. The PE methods used were pairwise ranking and matrix scoring.

3.2.2.1 Questionnaire survey

A questionnaire was administered in face-to-face interviews using local languages of the communities (*Kaffinono* in Bonga and *Afan Oromoo* in Horro). A total of 240 households were interviewed from two types of communities (CBBP, n = 60 and non CBBP, n = 60) in each of the study locations. Having explained the objectives of the study and obtained verbal consent for willingness to participate, the respondents were asked to list the major health problems of sheep in their community and rank them in order of importance. Afterwards, local knowledge questionnaire regarding internal parasites with particular emphasis on GIN was followed. Color pictures of six helminth parasites of sheep representing nematodes, cestodes and trematodes were shown on a laminated A4 paper (supplementary material 1). The respondents were asked whether or not they had ever seen them, and if yes, they were asked to tell the local names, location in sheep, and perceived disease caused by each. Follow up knowledge questions asked included perceived transmission and harmful effects of GIN on sheep. Besides, aspects of ethnoveterinary practices, mainly medicinal plants (local names, parts, etc.) used in control of GIN of sheep were asked. The medicinal plants were identified based on literature (Awas and Demissew, 2009). Furthermore, anthelmintic practice questions were asked by presenting anthelmintic boli available in local markets (albendazole bolus, etc.) to facilitate interviews, such as on the type and frequency of use over the past one year. Finally, preference data were collected based on four methods for control of GIN of sheep; anthelmintics, ethnoveterinary medicine, selective breeding and nutritional supplementation. These were rated for four attributes, each on a 5-point scale as follows: effectiveness (1 = not effective at all to 5 = very effective); cost (1 = very expensive to 5 = very cheap); availability (1 = hardly available to 5 = easily available); sustainability (1 = not sustainable to 5 = very sustainable). The scales were formulated by extending possible binary response category (yes/no) for an attribute of a GIN control option in literature (e.g, Stear et al., 2007; 2010). For instance, a questionnaire that can be designed as whether anthelmintics are effective or not, was modified as how effective are anthelmintics for control of GIN. This allowed more response categories (1 = not effective at all, 2 = not effective, 3 = moderately effective, 4 = effective, 5 = very effective).The attributes were explained verbally when required, sustainability, for instance, as the use of a control method for GIN in sheep at present without compromising its future (Waller, 2006; Stear, 2010).

#### 3.2.2.2 Pairwise ranking

This was used to identify and prioritize main disease problems of sheep in CBBP. Five most important sheep disease conditions with respect to their occurrence and severity were identified and drawn on cards to facilitate communication with illiterate farmers. The simplified pairwise ranking was adapted from Russell (1997) whereby seeds were used to score the relative importance of the diseases. The greater the number of seeds piled, the more important the disease (please see Supplementary material 2a-b). The total number of seeds (N) equaled the number of pairs of disease conditions compared, was determined using N = n (n-1)1/2, where n is the number of disease conditions. Four pairwise rankings were done; two in each CBBP of Bonga and Horro, separately for men and women groups. The number of participants ranged from five to seven per ranking group.

#### 3.2.2.3 Matrix scoring

Matrix scoring was used to analyze GIN control preferences within CBBP with the same groups of farmers. It was adapted from the methods described by Catley et al. (2012; 2001). Drawings representing five major disease conditions of sheep, as for pairwise ranking, were displayed along the y-axis. Four GIN control methods in sheep (ethnoveterinary, anthelmintic, nutritional supplementation and selective breeding) were displayed along the x-axis. These were represented by real objects except, selective breeding that was represented by two contrasting drawings of sheep for worm burden in stomach (low vs. high) (please see Supplementary material 3a-b). For each disease condition, 20 seeds were provided, and the informants were asked to divide the seeds from 0-20 over the control options, indicating that the higher the number of seeds the more the use of a control option for that disease condition. The number of seeds was chosen assuming that five seeds per disease control option are sufficient to indicate differences between these options. In total, four matrix scorings were done (two per location), separately for men and women. After completing a matrix, participants were interviewed about their choices using a Semi Structured Interview (SSI).

### 3.2.3 Genomic data

#### 3.2.3.1 Data source, sampling and genotyping

SNP genotype data for 110 Bonga sheep were obtained from International Center for Agricultural Research in the Dry Areas (ICARDA), Ethiopia. The animals were sampled from three villages implementing CBBP in Bonga (Boka, n = 61; Buta, n = 13; and Shuta, n = 36). Buta is one of the scaled out CBBP in Bonga, while Boka and Shuta are the founding CBBPs.

Blood samples were collected from individual animals and DNA was extracted following standard procedures at International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. Genotyping was performed by GeneSeek, Inc. (a Neogene company, Lincoln, NE, USA) with Illumina OvineHD BeadChip, which includes 606,006 SNPs. Additionally, SNP data genotyped with Ovine SNP50 BeadChip (50K) for three other Ethiopian sheep were kindly provided by OvineHapMap (Menz, n = 34) and Dr. Zewdu Edea (Blackhead Somali, n = 15; Horro, n = 15 and Menz, n = 12), cf. Edea et al. (2017).

3.2.3.2 Data preparation and quality control (QC)

Standard PLINK files were prepared using R (R Core Team, 2017). QC was done with the software PLINK 1.9 (Purcell et al., 2007). SNPs and individuals with a genotyping call rate < 90% were excluded. SNPs (with) minor allele frequency (MAF) < 0.05, deviating from Hardy Weinberg equilibrium (HWE) at $p < 10^{-6}$ and those unmapped and non-autosomal were excluded. After QC was performed, 107 animals in villages of Boka (n = 59), Buta (n = 13) and Shuta (n = 35) and 427,237 SNPs were retained for ROH analysis of the Bonga CBBP populations. When the HD and the 50K datasets were merged and checked for quality, 28,043 SNPs from 175 animals were available for analysis of population structure.

3.2.3.3 Detection of runs of homozygosity (ROH)

ROH were detected for each animal using PLINK 1.9 (Purcell et al., 2007). The following parameters and thresholds were applied: (i) the minimum length to define a ROH was set to be 670 kb; (ii) the minimum number of SNPs that a ROH required to have was set to be 20 SNPs; iii) the minimum density was set at 1 SNP per 50 kb; iv) the maximum gap between two consecutive SNPs in a ROH was set at 500 kb; and v) 1 heterozygous genotype and 1 missing genotype were permitted within each ROH. Considering that recombination rate in sheep is higher than other mammals (Johnston et al., 2016), mean = 1.5 cM/Mb (Petit et al., 2017), ROH were classified into five length categories: ROH > 0.67, > 1.33, > 3.33, > 6.67 and 11.11 Mb, corresponding to approximately 50, 25, 10, 5, and 3 generations since the common ancestor. The longest class of ROH relating to recent generations coincided with the establishment of CBBP for Bonga sheep.

### 3.3 Statistical analysis

#### 3.3.1 FEC and FAMACHA

FEC data was 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.

#### 3.3.2 Questionnaire and participatory epidemiology

Rankings for disease conditions of sheep within CBBP based on questionnaire were obtained by calculating indices, adapting a method applied by Zvinorova et al. (2017), as follows:

$$\text{Index} = \frac{\sum(3 \text{ for rank } 1 + 2 \text{ for rank } 2 + 1 \text{ for rank } 3) \text{ for a disease condition}}{\sum(3 \text{ for rank } 1 + 2 \text{ for rank } 2 + 1 \text{ for rank } 3) \text{ for all disease conditions}}$$

Also, weighted rankings of the disease conditions were done via triangulation of questionnaire rankings with pairwise rankings, to better understand the relative importance of GIN related problem within the CBBP. These were obtained by computing weighted mean ranks from questionnaire ranks and men's and women's pairwise rankings. Catley et al. (2012) suggest across method triangulation, the use of more than one method to answer the same research question. GIN control methods based on matrix scoring and ranking were analyzed descriptively. Statistical analyses on questionnaire data were performed using SPSS (IBM SPSS Statistics 20, 2011) and SAS 9.4 (SAS Institute Inc., 2012). Pearson's chi-square ($\chi^2$) test, or, in cases when more than 20% of the cells had expected counts less than 5, Fisher's exact test was used to test differences between CBBP and non CBBP regarding knowledge of helminth parasites of sheep and use of ethnoveterinary medicinal plants in the control of GIN of sheep. Cochran–Mantel–Haenszel $\chi^2$ test was employed to find whether the differences in knowledge between CBBP and non CBBP varied between locations (Bonga and Horro). The Cochran–Mantel–Haenszel $\chi^2$ test was also used to determine associations between communities (CBBP or non CBBP) and frequencies of anthelmintic use for control of GIN/helminth parasites of sheep, while controlling for locations. Differences between communities involved and not involved in CBBP for preferences of control methods of GIN in sheep were analyzed using Mann-Whitney U test.

### 3.3.3 Genomic inbreeding coefficients ($F_{ROH}$) and fine-scale population structure

#### 3.3.3.1 Analysis of genomic inbreeding coefficients ($F_{ROH}$)

Genomic inbreeding coefficients ($F_{ROH}$) were estimated based on ROH, as described by McQuillan et al. (2008): $\Sigma\, L_{ROH}/\Sigma\, L_{auto}$, the ratio of the sum of ROH length of an individual animal to the total size of the autosomes covered by the SNPs (where the $\Sigma\, L_{auto}$ was 2450.1 Mb in this study). The GLM procedure and the LSMEANS statement (SAS Institute Inc., 2012) were used to analyze differences in inbreeding levels between the CBBP villages. The PDIFF option on the LSMEANS statement was applied to obtain pairwise t-tests for all pairs of least squares means (LSM) within the same length category of ROH.

#### 3.3.3.2 Fine-scale population structure analysis

Analysis of fine-scale population structure was performed using the NetView pipeline as described by Neuditschko et al. (2012) and Steining et al. (2016), implemented in R (https://github.com/esteinig/netviewR). The pipeline uses mutual k-nearest neighbor graphs (k-NN) for this purpose. In brief, the NetView analysis consists of the following 3 steps on quality checked data: calculation of distance matrix based on allele-sharing distances in PLINK by subtracting identity by state from one (1-IBS); construction of networks; visualization of the networks. Besides, analysis of multidimensional scaling (MDS) was performed for Bonga sheep in CBBP with other three sheep populations in Ethiopia as references, using PLINK, and results were visualized in R.

# 4 Results and discussion

## 4.1 Gastrointestinal nematode infections and associated factors under communal sheep breeding in Ethiopia

### 4.1.1 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 FEC values during the rainy season were 5.05 ± 0.07 (131.11 ± 9.38) and 4.91 ± 0.06 (110.02 ± 6.73).

**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 $p < 0.001$

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 (Aga et al., 2013; Haile et al., 2010) 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.

4.1.2 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 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.

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* spp (Wyk and Bath, 2002; Kaplan et al., 2004).

4.1.3 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 4(a-d)) for Bonga and Horro sheep during rainy and dry seasons.

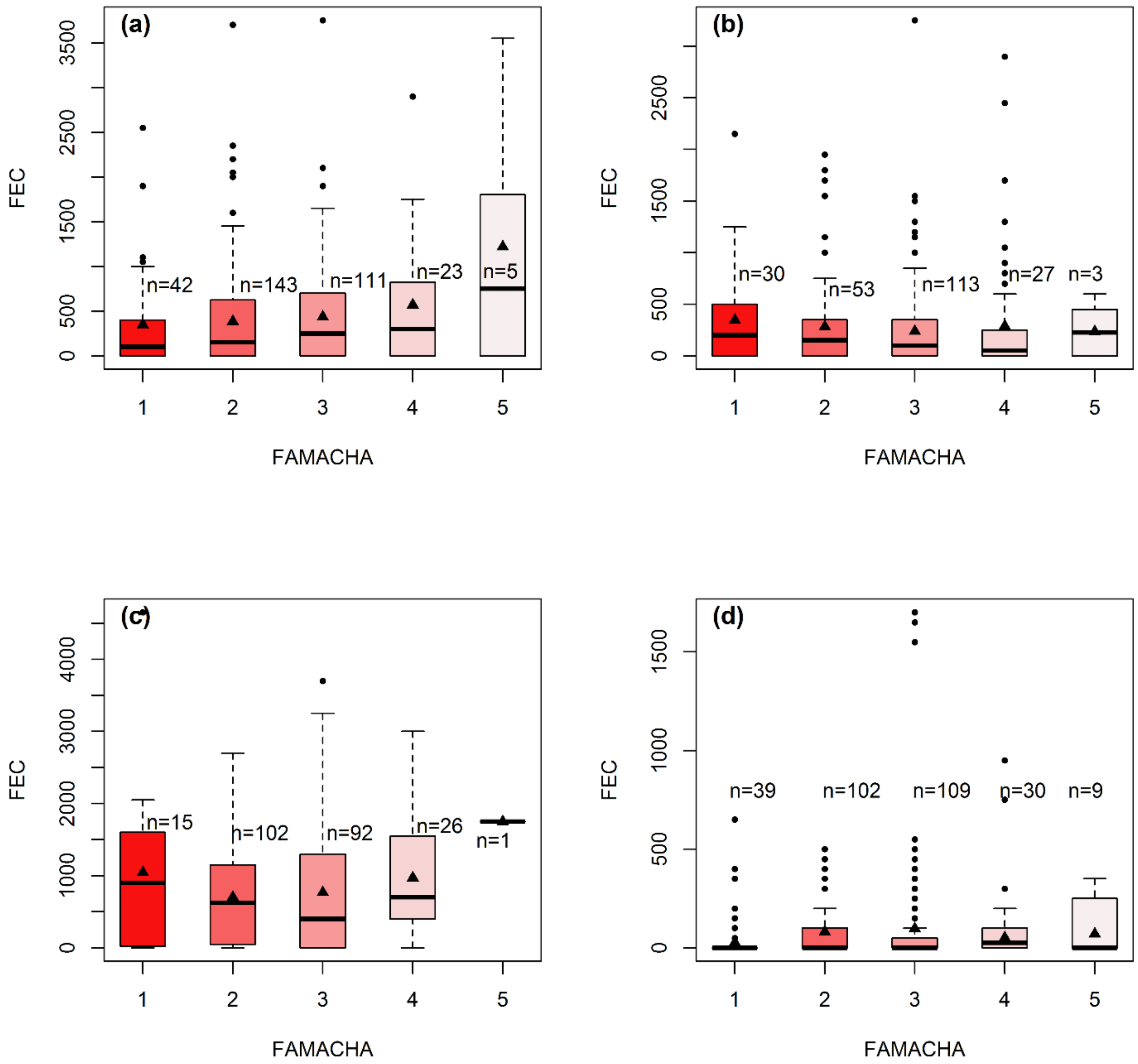



**Figure 4** Boxplots showing the relationship between FEC and FAMACHA scores in CBBP sheep: **(a)** Bonga, rainy season; **(b)** Horro, rainy season; **(c)** Bonga, dry season; **(d)** Horro, dry season. Solid triangles show the mean and solid lines show the median of FEC in each FAMACHA category. The 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.

There were no significant differences in FEC between FAMACHA classes: Kruskal-Wallis Test; $\chi^2 = 1.53$—$4.643$, 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).

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

| | FEC | | | | | |
|---|---|---|---|---|---|---|
| | Bonga | | | Horro | | |
| FAMACHA | 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 |

It appears that FAMACHA score increases 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 low 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.

## 4.2 Local knowledge, practices and preferences in control of GIN in Bonga and Horro sheep

### 4.2.1 Indigenous knowledge

#### 4.2.1.1 Identification of main health problems of sheep in CBBP

Results of questionnaire and pairwise ranking, and the weighted ranking of both methods are presented in Table 4 showing main disease problems of sheep in Bonga and Horro CBBP.

**Table 4** Rankings of disease conditions in sheep of Bonga and Horro CBBP.

| disease conditions | questionnaire | | pairwise ranking | | | | weighted | |
|---|---|---|---|---|---|---|---|---|
| | | | Men | | Women | | | |
| | Index | rank | score | rank | score | rank | mean | rank |
| **Bonga CBBP** | | | | | | | | |
| bottle jaw | 0.14 | 4 | 0 | 5 | 1 | 4 | 4.3 | 4 |
| coughing | 0.11 | 5 | 1 | 4 | 2 | 3 | 4 | 3 |
| coenurosis [a] | 0.31 | 1 | 3 | 2 | 4 | 1 | 1.3 | 1 |
| diarrhea | 0.17 | 3 | 4 | 1 | 3 | 2 | 2 | 2 |
| weight loss | 0.01 | 6 | 2 | 3 | 0 | 5 | 4.7 | 5 |
| Others [b] | 0.26 | 2 | - | - | - | - | - | - |
| Total | 1 | | 10 | | 10 | | | |
| **Horro CBBP** | | | | | | | | |
| bottle jaw | 0.32 | 1 | 2 | 4 | 1 | 5 | 3.3 | 3 |
| coughing | 0.25 | 2 | 5 | 1 | 4 | 1 | 1.3 | 1 |
| coenurosis [a] | 0.06 | 5 | 1 | 5 | 2 | 4 | 4.7 | 6 |
| diarrhea | 0.21 | 3 | 3 | 2 | 4 | 1 | 2 | 2 |
| weight loss | 0.02 | 6 | 3 | 2 | 1 | 5 | 4.3 | 5 |
| Others[b] | 0.14 | 4 | 1 | 5 | 3 | 3 | 4 | 4 |
| Total | 1 | | 15 | | 15 | | | |

[a] coenurosis: circling movement of affected individuals, caused by *coenurus cerebralis*

[b] disease conditions; eye problem, ascites (fluid accumulation in the abdominal cavity), sore mouth caused by orf virus, external parasites, etc.

The lowest rank indicates the most important.

Questionnaire ranking showed that the most important (1$^{st}$, 2$^{nd}$ and 3$^{rd}$) disease conditions of sheep in Bonga CBBP were coenurosis, others (category of disease conditions, such as eye problem, sore mouth caused by orf virus , etc.) and diarrhea; while in Horro CBBP these were bottle jaw, coughing and diarrhea. When weighted ranking was considered the most important disease conditions of sheep in CBBP were coenurosis, diarrhea and coughing in Bonga, whereas coughing, diarrhea and bottle jaw were ranked 1st to 3rd in Horro.

According to Schillhorn van Veen (1997), ethnosemantic diagnoses of parasitic diseases are based on signs. For instance, livestock farmers in Mexico consider bottle jaw, a disease by itself. Similarly, in our study, sheep farmers named diseases based on signs. A weighted ranking demonstrated that coughing followed by diarrhea and bottle jaw in Horro, and diarrhea next to coenurosis in Bonga were the main health problems in CBBP sheep. Diarrhea and bottle jaw may be attributed to GIN infections (Schillhorn van Veen, 1997; Bath and van Wyk, 2009; Idris et al., 2012). Our finding apparently agrees with high prevalence of GIN infection reported in sheep of Horro (Regassa et al., 2006) and Bonga (Desta, 2015).

#### 4.2.1.2 Local knowledge of GIN and other internal parasites of sheep

Table 5 shows farmers' perceptions about GIN and other internal parasites of sheep. These include perceived transmission, signs/harmful effects, morphology and local names based on pictures of adult helminth parasites shown during interview. In Horro, there was highly significant difference ($\chi2 = 18.4$, $p < 0.0001$) between CBBP (90%) and non CBBP (55%) respondents who reported that GIN infect sheep during grazing. In Bonga, CBBP communities also had significantly better knowledge about transmission of GIN infection than non CBBP communities ($\chi2 = 4.2$, $p < 0.05$; 95% vs 83%, respectively). Yet, the Cochran-Mantel-Haenszel test (CMH = 21.9, $P < 0.0001$) indicated that this difference in knowledge between CBBP and non CBBP communities was significantly larger in Horro than in Bonga. Almost all respondents (96.7 and 100% CBBP and non CBBP in Horro; Fisher's exact test, $p > 0.05$) and 100% CBBP and non CBBP in Bonga perceived that stomach and intestinal worms are harmful to sheep. The reported harmful effects of GIN on sheep were slow growth in lambs, diarrhea, loss of weight, and often death. Only very few respondents in Horro (3.3% CBBP and non CBBP; Fisher's exact test, $p > 0.05$) reported that they saw adult GIN parasites (e.g., *Haemonchus* spp) in sheep during slaughtering at backyard. However, they did not tell local names. Similar observation was reported in Bonga (1.7 CBBP and 7.5% non CBBP; Fisher's exact test, $p > 0.05$). Nearly a quarter of respondents (31 and 20% CBBP and non CBBP; $\chi2$ = 1.9, $p > 0.05$) in Horro reported that the local name of liver fluke is *jiitoo* or *baallee*. In Bonga, very few respondents (8.3% CBBP and 5% non CBBP; Fisher's exact test, $p > 0.05$) mentioned the local name of liver fluke. However, they named it inconsistently. A majority of respondents saw helminth in faeces of sheep (*Moniezia* spp) and there was no significant difference between non CBBP and CBBP communities (45.6 and 62.1% Horro $\chi2$ = 3.1, $p > 0.05$; 85.0 and 87.5% Bonga, $\chi2$ = 0.2, $p > 0.05$).

**Table 5** Farmers' perception of GIN and other internal parasites of sheep among communities of Horro and Bonga.

| Knowledge questions | | Horro | | Bonga | | CMH [b] test, comparing CBBP and non CBBP across locations | descriptions of knowledge of GIN and other helminths |
|---|---|---|---|---|---|---|---|
| | | non CBBP (n = 60) [a] | CBBP (n = 60) | non CBBP (n = 60) | CBBP (n = 60) | | |
| **Tapeworms** | | | | | | | |
| Ever seen helminths in | yes (%) | 45.6 | 62.1 | 85.0 | 87.5 | | respondent has seen *Moneizia* spp (color plate shown |
| faeces of sheep? | Significance [c] | ns | | ns | | ns | during interview) |
| **Flukes** | | | | | | | |
| Ever seen helminths in | yes (%) | 43.3 | 68.3 | 45 | 48.3 | | respondent has seen rumen fluke (color plate shown) |
| rumen/stomach of sheep? | Significance | ** | | ns | | * | |
| Ever seen helminths in | yes (%) | 28.3 | 51.7 | 12.5 | 17.5 | | respondent has seen liver fluke (color plate shown) |
| liver of sheep? | Significance | ** | | ns | | ** | |
| Know local name of | yes (%) | 20 | 31 | 5 | 8.3 | | respondent named *jiitoo* or *baallee* in Afan Oromoo, |
| liver fluke | Significance | ns | | ns | | ns | name mentioned—inconsistently in kaffinono |
| **Roundworms** | | | | | | | |
| Ever seen helminths in | yes (%) | 3.3 | 3.3 | 7.6 | 1.7 | | respondent has seen *Haemonchus* spp or Trichuris spp |
| stomach/intestine of sheep? | Significance | ns | | ns | | ns | (color plates shown) |
| Know helminths are | yes (%) | 96.7 | 100.0 | 100.0 | 100.0 | | respondent named two or more harmful effects of |
| harmful? | Significance | Ns | | - | | ns | GIN; weight loss/slow growth rate in lambs, diarrhea, anaemia, death |
| Know helminths transmission | yes (%) | 55 | 90 | 83.3 | 95 | | respondent linked transmission of GIN with ingestion |
| | Significance | *** | | * | | *** | of (infected) grass |

[a] Number of observations are lower for some questionnaire (per the communities, n < 60) due to missingness.

[b] Cochran–Mantel–Haenszel Statistics

[c] Pearson's $\chi 2$ test with degrees of freedom 1, or Fisher's exact test and significance levels are presented as: ns = P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001.

CBBP communities were more knowledgeable about helminth parasites of sheep. This may be attributed to control practices of helminth parasites embedded in the breeding program. Our study also showed that most farmers in Bonga and Horro are aware of the transmission of GIN in sheep. This is in contrast with the study of Zvinorova et al. (2017) who reported that the majority of farmers in Zimbabwe lack knowledge on spread of GIN in small ruminants. The *Afan Oromoo* names of liver fluke, *jiitoo* or *baallee*, literally mean wet or leaf-shaped, are associated with the aspects of epidemiology and morphology (Urquhart et al., 1996), respectively, an infection with the liver fluke when sheep graze on wetland/swampy areas and the leaf-shaped appearance of the parasite. Farmers' observation on adult internal parasites of sheep (at home slaughtered animals) was better for flat worms than round worms. This could be due to the fact that farmers are more likely to experience knowledge of parasites residing in edible organs (e.g. liver—*Fasciola* spp) than non-edible ones (e.g. abomasum—*Haemonchus* spp).

4.2.2 GIN control practices

4.2.2.1 Ethnoveterinary practices in control of GIN of sheep

Almost all (97.5%) farmers in Horro (both CBBP and non CBBP) did not use ethnoveterinary method for control of GIN of sheep. In Bonga, however, they use ethnoveterinary medicinal plants for the control of GIN of sheep (Table 6). The most frequently used plant for control of GIN in Bonga sheep was *Ocimum lamiifolium*, cited by 38.3 and 35.0% respondents in non CBBP and CBBP communities. There was no significant difference ($p > 0.05$) between the communities in the use citations. The second and the third most reported medicinal plants were *Nicotiana tabacum* (33.3% non CBBP vs 20.0% CBBP; $p > 0.05$) and *Pycnostachys abyssinica* (30.0% CBBP vs 10.0% non CBBP; $p < 0.01$).

**Table 6** Ethnoveterinary medicinal plants used in control of GIN of sheep in Bonga.

| Plants' local name | Scientific name | parts used | preparation (administration) | use citations, n (%) | | p-value[1] |
|---|---|---|---|---|---|---|
| | | | | non CBBP | CBBP | |
| Kosho | *Hagenia abyssinica* | leaves, flowers | drenching | 3 (5.0) | 9 (15.0) | ns |
| Tumbao | *Nicotiana tabacum* | Leaves | oral (drenching), nasal | 20 (33.3) | 12 (20.0) | ns |
| Damo | *Ocimum lamiifolium* | Leaves | mixed with feed, drenching | 21 (35.0) | 23 (38.3) | ns |
| Shinatto | *Arundinaria alpine* | Leaves | drenching | 5 (8.3) | 5 (8.3) | ns |
| Yemesho | *Bothriocline schimperi* | Leaves | drenching | 2 (3.3) | 5 (8.3) | ns |
| Yearo | *Pycnostachys abyssinica* | Leaves | drenching, mixed with feed | 6 (10.0) | 18 (30.0) | ** |
| Wago | *Croton macrostachyus* | Leaves | drenching | 2 (3.3) | 12 (20.0) | ** |
| Kaphero | *Echinops kebericho* | Roots | decoction, smoking | 2 (3.3) | 8 (13.3) | ns |

[1] Fisher's exact (or $\chi2$) test: ns = P > 0.05; *P < 0.05; **P < 0.01.

In addition, three plants with local names frequently mentioned were Ataro (15% non CBBP vs 0% CBBP; Fisher's exact test, p < 0.01), Okato (13.3% non CBBP vs 8.33% CBBP; $\chi2$ test, p > 0.05) and Manjecho (10% CBBP vs 2.3% non CBBP; $\chi2$ test, p > 0.05).

In Horro communities, ethnoveterinary knowledge for control of GIN of sheep is not practiced. This could in part be attributed to the heavy reliance on anthelmintic use in the communities resulting in replacement of ethnoveterinary practice. Consequently, the ethnoveterinary knowledge could be lost because it is passed orally from generation to generation, as this information is not documented and remains in the memory of elderly practitioners (Awas and Demissew, 2009). But, Bonga communities have maintained good knowledge of using medicinal plants for control of GIN in sheep. They use diverse plants, the use of some of which like tobacco leaves (*Nicotiana tabacum*) against nematodes has not been reported in Ethiopia. Interestingly, however, in other parts of developing countries, for instance, in India, in vitro anthelmintic effect of tobacco extract on parasitic nematode, *Marshallagia marshalli*, was found to be as effective as a standard anthelmintic levamisole (Nouri et al., 2016). Similarly, Iqbal et al. (2006) found the effectiveness of the extracts of leaves of tobacco against nematodes and concluded that their use is justifiable in traditional medicine system of Pakistan. The reminder of medicinal plants reported here should be verified for their efficacy against nematodes, since in some cases plants may have been mistakenly included in lists with those reported with anthelmintic properties and these mistakes may be justified when controlled experimentation is performed (Athanasiadou, et al., 2007).

4.2.2.2 Anthelmintic practices in control of sheep GIN within CBBP and non CBBP

Table 7 presents anthelmintic use and community type, by location for control of GIN and other internal parasites of sheep. Results indicate significant difference ($p < 0.001$) in frequency of anthelmintic use between CBBP and non CBBP communities for each of the two locations. A global Cochran-Mantel-Haenszel test confirmed that this difference is also significant (Cochran–Mantel–Haenszel Statistics = 29.6, df = 1, $p < 0.001$) after controlling for locations.

**Table 7** Anthelmintic use and community type, by location for control of GIN and other internal parasites of sheep.

| | | anthelmintic use (% respondents)[1] | | | | Mantel-Haenszel $\chi 2$ | | |
|---|---|---|---|---|---|---|---|---|
| location | community | ≤ once | twice | thrice | ≥4 times | value | df | p-value |
| Bonga | CBBP (n = 60) | 8.3 | 5.0 | 31.7 | 55.0 | 11.1 | 1 | *** |
| | non CBBP (n = 60) | 5..0 | 25.0 | 55.0 | 15.0 | | | |
| Horro | CBBP (n = 60) | 0.0 | 0.0 | 15.0 | 85.0 | 19.5 | 1 | *** |
| | non CBBP (n = 60) | 10.0 | 13.3 | 25.0 | 51.7 | | | |

[1] Percentage of respondents who treated their sheep: none or once, twice, thrice, four times or greater over the last one year.

***P < 0.001.

The extent of anthelmintic use in our finding is higher than the study of Datiko et al. (2013) who reported the maximum number of two treatments per year in and around Bishoftu, central Ethiopia. Besides, there is a clear indication that CBBP farmers practice utilization of anthelmintic for control of GIN and other helminth parasites of sheep more frequently than the non CBBP farmers. This heavy reliance on anthelmintic may lead to development of resistance to anthelmintic in the CBBP communities' sheep. This suggests that introduction of more sustainable control options for GIN of sheep is important in these communities. However, farmers' positive attitude toward anthelmintics use, which in turn developed from their current antheminics use, may be a barrier for possible uptake of sustainable control method, such as genetic selection in CBBP (Vande Velde et al., 2018).

### 4.2.3 Preferences of GIN control

#### 4.2.3.1 Preferences for GIN control options in CBBP and non CBBP: questionnaire

Preferences of CBBP and non CBBP farmers for sheep GIN control options and their attributes in Bonga and Horro are provided in Table 8. Ethnoveterinary control was rated not effective for GIN of sheep by Horro CBBP and non CBBP farmers though there was highly significant difference in degree of their rating (Mann-Whitney U test: $p < 0.01$; median = 1 for each community, interquartile range (IQR) = 0 for CBBP and 1 for non CBBP). In Bonga, however, ethnoveterinary control was rated effective by both CBBP and non CBBP farmers, but there was a significant difference between their rating ($p < 0.05$). In both Horro and Bonga, farmers in CBBP and non CBBP did not differ in rating the cost of anthelmintic. They rated the cost as cheap (Mann-Whitney U test: $p > 0.05$; median = 4, IQR = 1, for both communities). This suggests that they tend to prefer anthelmintic in control of GIN of sheep due to its low cost.

**Table 8** Preferences of farmers in Bonga and Horro who are involved and not involved in community-based breeding programs (CBBP and non CBBP) for options of gastrointestinal nematodes (GIN) control.

| GIN control options[a] and attributes | | Horro | | | | | Bonga | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CBBP | | non CBBP | | | CBBP | | non CBBP | | |
| | | Mean | Median [IQR[b]] | Mean | Median [IQR] | p-value[U] | Mean | Median [IQR] | Mean | Median [IQR] | p-value |
| effectiveness | EV | 1.37 | 1 [0] | 1.76 | 1 [1] | ** | 4.12 | 4 [1] | 3.88 | 4 [0] | ns |
| | AH | 4.69 | 5 [0] | 4.41 | 4.5 [1] | ** | 4.83 | 5 [0] | 4.93 | 5 [0] | ns |
| | SB | 4.07 | 4 [1] | 2.45 | 3 [3] | *** | 2.92 | 3 [2] | 2.5 | 2.5 [3] | ns |
| | NS | 3.54 | 4 [1] | 2.53 | 2.5 [3] | *** | 3.03 | 5 [0] | 2.6 | 5 [0] | ns |
| availability | EV | 1.27 | 1 [0] | 1.55 | 1 [1] | * | 4.57 | 5 [1] | 4.05 | 5 [2] | * |
| | AH | 4.9 | 5 [0] | 4.74 | 5 [1] | * | 4.73 | 5 [1] | 4.31 | 4 [1] | *** |
| | SB | 2.9 | 3 [2] | 1.62 | 1 [1] | *** | 1.57 | 1 [1] | 1.67 | 1 [1] | ns |
| | NS | 2.08 | 2 [2] | 2.02 | 2 [1] | ns | 1.47 | 1 [0.5] | 1.34 | 1 [1] | ns |
| (cheap) cost | EV | 1.47 | 1 [0] | 2.03 | 1 [2] | * | 4.57 | 5 [0] | 4.28 | 5 [1] | ns |
| | AH | 3.59 | 4 [1] | 3.66 | 4 [1] | ns | 4.02 | 4 [1] | 4.19 | 4 [1] | ns |
| | SB | 3.12 | 3 [2] | 2.26 | 2 [2] | *** | 1.2 | 1 [0] | 1.64 | 1 [1] | * |
| | NS | 2.56 | 2 [3] | 2.19 | 2 [2] | ns | 1.4 | 1 [1] | 1.71 | 1 [1] | ns |
| sustainability | EV | 1.14 | 1 [0] | 1.55 | 1 [1] | ** | 3.75 | 4 [2] | 3.66 | 4 [1] | * |
| | AH | 4.76 | 5 [0] | 4.24 | 4 [1] | *** | 4.7 | 5 [0] | 4.84 | 5 [0] | ns |
| | SB | 4.03 | 4 [0] | 2.43 | 2 [3] | *** | 3.5 | 4 [1] | 3.57 | 4 [1] | ns |
| | NS | 2.97 | 3 [2] | 2.22 | 2 [2] | ** | 3.3 | 4 [2] | 3.53 | 4 [1] | ns |

[a] AH, anthelmintic; EV, ethnoveterinary; NS, nutritional supplementation (NS); and SB, selective breeding.

[b] Interquartile range

[u] Mann-Whitney U Test: P > 0.05; *P < 0.05; **P < 0.01; *** p < 0.001.

In Horro, CBBP farmers preferred selective breeding as a sustainable GIN control option more than non CBBP farmers, and the difference was highly significant ($p < 0.001$). This indicates that selective breeding has potential to be introduced into the CBBP. Also, CBBP farmers in Horro rated anthelmintic as a sustainable option for control of GIN significantly higher than non CBBP farmers ($p < 0.001$). However, this perception held by CBBP farmers indicates that they lack awareness of anthelmintic resistance.

Selective breeding for control of sheep GIN in Horro was favored more by CBBP than non CBBP farmers pertaining to its effectiveness and sustainability. Anthelmintics were the most preferred option in control of GIN of sheep across the CBBP and non CBBP for the criteria considered, including sustainability. However, the farmers' perceived sustainability of anthelmintic in control of GIN coupled with increased frequency of anthelmintic use in CBBP could lead to development of anthelmintic resistance. This indicates that anthelmintics are not a sustainable option. Besides, a single GIN control method may not be sustainable (Waller, 2006), suggesting a need to integrate different alternatives of GIN control methods.

#### 4.2.3.2 Preferences for GIN control options within CBBP: matrix scoring

As a complementary to preferences based on questionnaire, matrix scorings for preferences of GIN control methods were performed within CBBP (Table 9). In Bonga CBBP, men's group preferred anthelmintic as the most used method against bottle jaw, allocating a score value of 11 out of 20 seeds followed by ethnoveterinary (6/20 seeds), while women's group completely preferred anthelmintic for the same disease condition by assigning all the 20 seeds. In Horro CBBP, men had similar preference to Bonga regarding the use of anthelmintic against bottle jaw (11/20 seeds), followed by selective breeding (6/20 seeds). Women in Horro CBBP preferred nutritional supplementation as the most (12/20 seeds) preferred option for control of bottle jaw followed by anthelmintic (8/20 seeds). Neither men nor women associated GIN control methods with coenurosis in both CBBP areas.

**Table 9** Matrix scoring of disease conditions and control methods in sheep, preferences of the options by men and women in CBBPs of Bonga and Horro.

| | | scores for disease control options [a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Men | | | | | women | | | | |
| Bonga CBBP | disease condition | EV | AH | SB | NS | total | EV | AH | SB | NS | total |
| | bottle jaw | 6 | 11 | 1 | 2 | 20 | 0 | 20 | 0 | 0 | 20 |
| | weight loss | 10 | 4 | 1 | 5 | 20 | 10 | 8 | 0 | 2 | 20 |
| | Cough | 12 | 5 | 1 | 2 | 20 | 10 | 7 | 0 | 3 | 20 |
| | Diarrhea | 10 | 6 | 1 | 3 | 20 | 5 | 11 | 0 | 4 | 20 |
| | Coenurosis | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 20 |
| Horro CBBP | | | | | | | | | | | |
| | bottle jaw | 0 | 11 | 6 | 3 | 20 | 0 | 8 | 0 | 12 | 20 |
| | weight loss | 0 | 7 | 3 | 10 | 20 | 0 | 8 | 0 | 12 | 20 |
| | Cough | 2 | 10 | 3 | 5 | 20 | 0 | 13 | 0 | 7 | 20 |
| | Diarrhea | 0 | 10 | 4 | 6 | 20 | 0 | 10 | 3 | 7 | 20 |
| | Coenurosis | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 20 |

[a] AH, anthelmintic; EV, ethnoveterinary; NS, nutritional supplementation (NS); and SB, selective breeding.

Results of matrix scoring indicated that farmers associated methods of GIN control with signs of GIN infection. Interestingly, they did not associate these methods with coenurosis in both CBBP areas. Coenurosis is caused by Coenurus cerebralis (Urquhart et al., 1996), which is a non-GIN cause; hence none of the GIN control methods are valid against it. SSI on the results of matrix scoring showed that farmers would rather slaughter than use GIN treatment options when their sheep suffered from coenurosis. Similarly, when they were asked why they did not use SB for a majority of the health problems of sheep in their CBBP (in particular for GIN related signs), they said that because we don't have a way to identify whether or not our sheep harbor a large number of worms in their stomach/intestine, and also there is no veterinary laboratory which provides such service in our locality. This concords with limitation of selective breeding in literature (Stear et al., 2007) that it requires a high level of expertise though it is effective and inexpensive method of GIN control.

## 4.3 Genomic inbreeding and fine-scale population structure of Ethiopian sheep populations currently managed in communal breeding schemes

### 4.3.1 Inbreeding levels of Bonga sheep populations in CBBP

Table 10 shows least square means and standard errors (LSM ± SE) of $F_{ROH}$ for five length classes of ROH for Bonga sheep sampled from three CBBP villages. The LSM ± SE of $F_{ROH}$ for the minimum length of ROH regions ($F_{ROH > 0.67 Mb}$) are 0.068 ± 0.005, 0.060 ± 0.011 and 0.060 ± 0.007, respectively, in Boka, Buta and Shuta. The CBBP villages did not differ ($p > 0.05$) in the $F_{ROH}$ for all ROH length categories considered. This suggests similarity in breeding practices of the CBBP villages regarding control of inbreeding in former times as well as recently.

**Table 10** Least square means and standard errors (LSM ± SE) of genomic inbreeding coefficients ($F_{ROH}$) for five length categories of runs of homozygosity (ROH) for Bonga sheep sampled from three community-based breeding program (CBBP) villages.

| Effect | LSM ± SE of $F_{ROH}$ for different ROH length classes | | | | |
|---|---|---|---|---|---|
| | $F_{ROH > 0.67 Mb}$ | $F_{ROH > 1.33 Mb}$ | $F_{ROH > 3.33 Mb}$ | $F_{ROH > 6.67 Mb}$ | $F_{ROH > 11.11 Mb}$ |
| CBBP (N = 107) | Ns | Ns | Ns | ns | ns |
| Boka (n = 59) | 0.068 ± 0.005 | 0.033 ± 0.005 | 0.016 ± 0.005 | 0.013 ± 0.005 | 0.010 ± 0.004 |
| Buta (n = 13) | 0.060 ± 0.011 | 0.024 ± 0.011 | 0.008 ± 0.010 | 0.004 ± 0.011 | 0.002 ± 0.008 |
| Shuta (n = 35) | 0.060 ± 0.007 | 0.026± 0.007 | 0.011 ± 0.006 | 0.007 ± 0.006 | 0.005 ± 0.005 |

ns: not statistically significant ($p > 0.05$).

The inbreeding levels derived from ROH for Bonga sheep in CBBP are within the ranges reported for Italian sheep breeds. Of 25 Italian sheep breeds investigated, Mastrangelo et al. (2018) reported the highest value of inbreeding ($F_{ROH} = 0.099$) in Valle del Belice and the lowest ($F_{ROH} = 0.016$) in Comisana sheep. A study of ROH inbreeding levels across 31 populations of African goats (Nandolo et al., 2019) indicated a wide range of $F_{ROH}$ compared to our populations, averages ranging from 0.001 to 0.136, with a global average of 0.041, for $F_{ROH > 2 Mb}$.

4.3.2 Individuals with high inbreeding levels

Inbreeding levels of the most inbred sheep in Bonga CBBP populations representing inbreeding in the past 3 to 50 generations are presented in Figure 4 (a). These individuals (n = 7, $F_{ROH}$ > 0.10 for ROH > 0.67 Mb) are also depicted as outliers by box plots (Figure 4 (b)). The $F_{ROH}$ of these animals ranged from 0.111 to 0.313 for ROH > 0.67 Mb and from 0.032 to 0.232 for ROH > 11.11 Mb.

The most inbred individual (Boka_1), with $F_{ROH > 11.11 Mb}$ = 0.232 is probably the only one that is the product of parent-offspring or full-sib mating, for which an inbreeding coefficient of 0.25 is expected. This possibly resulted from mating of a sexually matured ram with its full-sib or its dam, before ram selection had been conducted. In CBBP, selected rams are moved between flocks and grazing areas to minimize this kind of inbreeding. The second most inbred individual showed $F_{ROH > 0.67 Mb}$ = 0.157, which is consistent with half-sib or offspring-grandparent mating. The third most inbred individual had $F_{ROH > 0.67 Mb}$ = 0.093, which is consistent with half-sib and first cousin mating. The ROH inbreeding coefficients related to very recent inbreeding ($F_{ROH > 11.11 Mb}$) of the four other highly inbred animals were below 0.07, excluding the possibility of half-sib mating. Comparing $F_{ROH}$ of different minimum lengths, levels of $F_{ROH > 0.67 Mb}$ were typically 0.05 to 0.06 higher compared to $F_{ROH > 11.11 Mb}$ (see Table 10 and Figure 5 (a)).

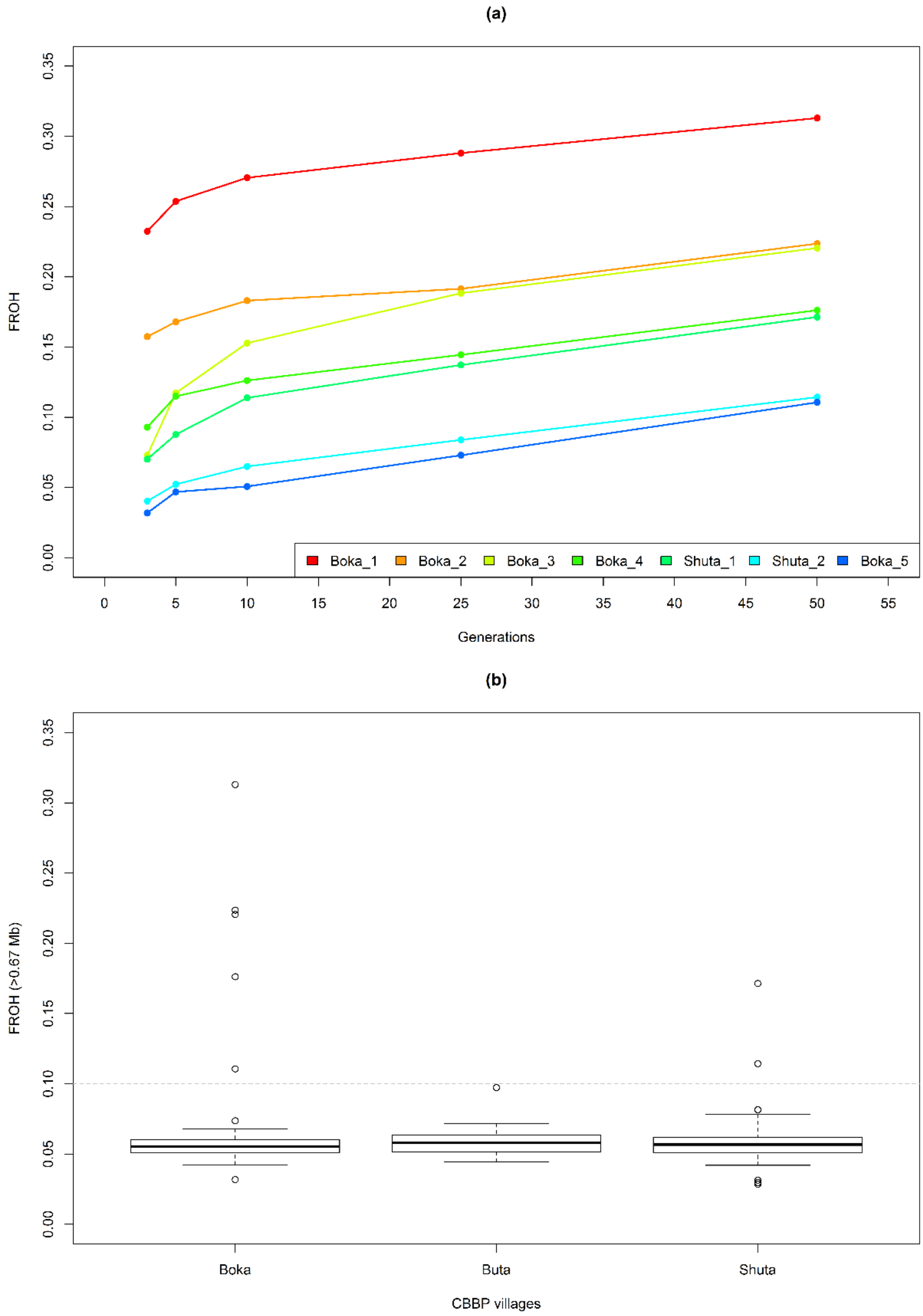



**Figure 5 (a)** Most inbred sheep from Bonga community-based breeding program (CBBP) villages (Boka, Buta and Shuta) in the past 3 to 50 generations, **(b)** Box plots showing the most inbred individuals as outliers (with genomic inbreeding coefficient, $F_{ROH}$ > 0.10 in the Bonga CBBP villages based on inbreeding generated 50 generations ago ($F_{ROH > 0.67\ Mb}$).

### 4.3.3 Network construction of Bonga sheep populations in CBBP

Figure 6 presents the network of Bonga sheep for three CBBP villages. The CBBP in two villages (Boka and Shuta) were established slightly earlier than in Buta. The interest was to see if there will be any substructure in the sheep populations of these three villages. The results indicated no structuring of Bonga sheep into subpopulations of CBBP villages. This most probably be due to the fact that the CBBP are still young and did not lead to genetic separation yet. Therefore, all the Bonga sheep samples from different villages may be considered as one population.

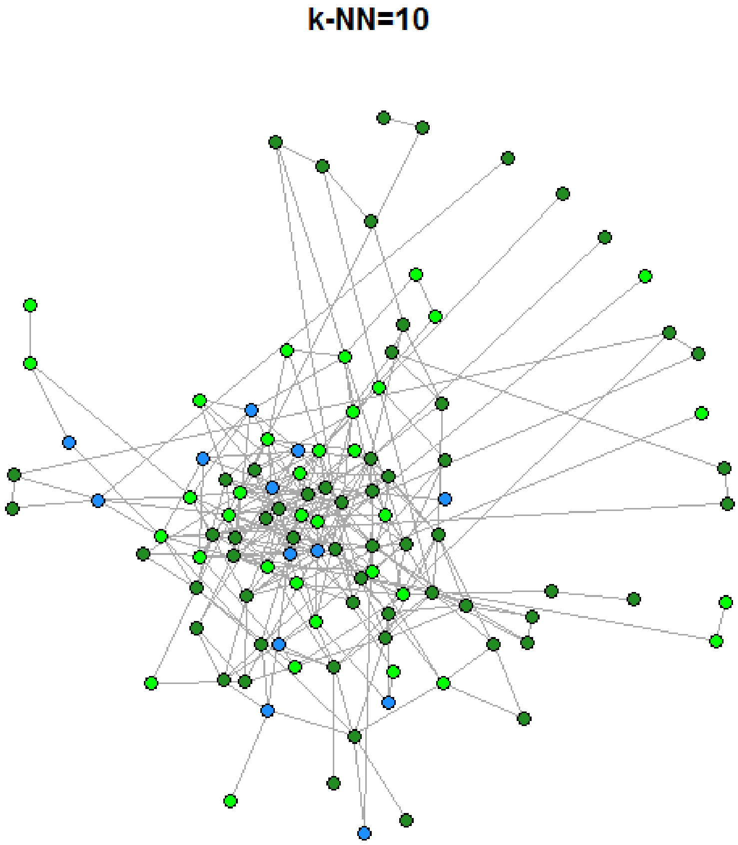



**Figure 6** Network visualization of Bonga sheep for three CBBP villages (Boka, dark green; Buta, light blue; Shuta, green). Construction of networks was done using k-NN = 10, the mutual k-nearest neighbors thresholds, as suggested by (Steining et al., 2016).

Multidimensional scaling (MDS) plot of Bonga sheep of the three CBBP villages, treated as one population, compared to three other Ethiopian sheep breeds is provided in Figure

7. The first and the second dimensions separated the populations into four distinct breeds. This is in line with previous studies of Gizaw et al. (2007) and Edea et al. (2017) who used microsatellite and high-density SNP markers, respectively, for genetic diversity and population structure of Ethiopian sheep populations. The close clustering pattern in Bonga sheep is notable. This corroborates with our results of NetView.

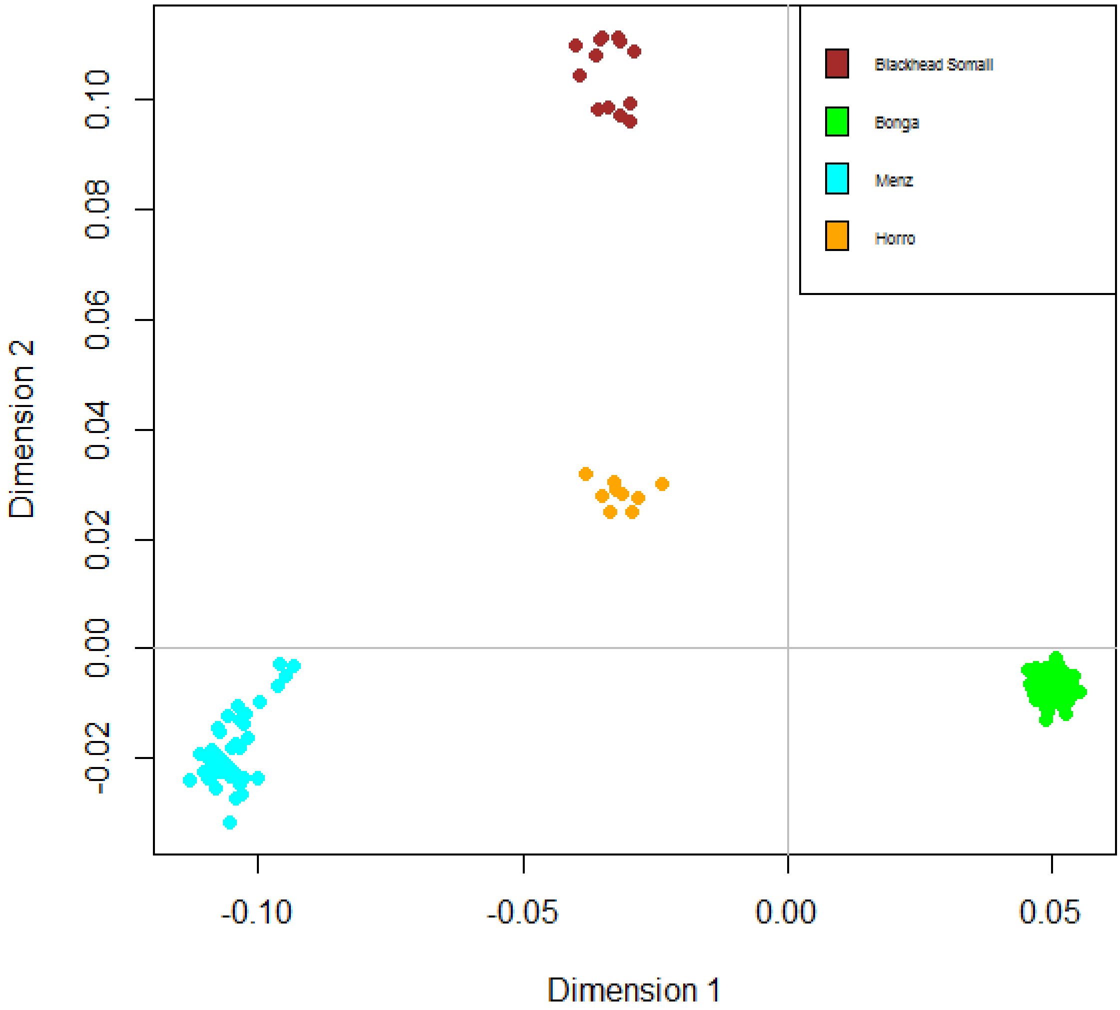



**Figure 7** Multidimensional scaling (MDS) plot of Bonga sheep for three CBBP villages (Boka, Buta and Shuta) with three other Ethiopian sheep breeds (Blackhead Somali, Menz and Horro) used as references.

## 5 General reflection and conclusions

Despite sheep are the second most important livestock species in Ethiopia, multiple factors hamper sheep productivity (Gizaw et al., 2013). Efforts to sheep breeding programs have failed, in part due to top-down approach for the development of breeding programs that overlooked the involvement of sheep keepers. As an alternative, community-based breeding programs (CBBP) have recently been designed and implemented for three Ethiopian sheep breeds, namely Bonga, Horro and Menz (Duguma, 2010). The CBBP as described by Mueller et al. (2015), are breeding programs typically related to low-input livestock production systems in developing countries involving farmers within limited geographical boundaries having a common interest to improve and share their genetic resources. Diseases and parasites like helminthosis (Biffa et al., 2006) are also among the constraints that constrained sheep production in the country. Gastrointestinal nematodes (GIN) are a problem of health, welfare and production in sheep populations worldwide (Mavrot et al., 2015; Roeber et al., 2013). They can cause parasitic gastroenteritis typically in young animals, which provokes clinical signs, such as diarrhea, reduced growth and weight loss (Vande Velde et al., 2018). According to Duguma (2010), the Ethiopian CBBP project introduced seasonal mass de-worming of animals. However, this is not sustainable due to increased risk of anthelmintic resistance, necessitating to look for sustainable helminth control options. In this regard, GIN resistance ought to be incorporated into the breeding goal traits of the CBBP. Inbreeding resulting from mating of individuals related by common ancestry (Blouin and Blouin, 1988) is an inevitable in populations under selection (Marras et al., 2015). It has shown to negatively affect birth weight, average daily gain, and litter size in sheep, consequently, it is an important parameter to monitor and control in breeding programs (Norberg and Sørensen, 2007).

Control of both GIN and inbreeding are important in sheep breeding programs, such as in CBBP of Ethiopia. However, prior to designing appropriate GIN and inbreeding control strategies, a better understanding of intensity and associated factors, local knowledge, current practices and preferences for different control options, and levels of inbreeding is needed. In this study the intensity of GIN infections and its relationship with level of anaemia; the indigenous knowledge, practices and control options of GIN; and the genomic inbreeding and fine-scale structure were investigated in Bonga and Horro sheep of Ethiopia, currently managed under communal breeding programs.

Section **4.1** of this thesis presented GIN infections and associated factors under communal sheep breeding in Ethiopia. A total of 1239 faecal egg count (FEC) and FAMACHA scores were measured on Bonga and Horro sheep during rainy and dry seasons to determine the intensity of GIN infections and its relationship with level of anaemia. The FEC and FAMACHA data were analyzed using mixed model procedures, accounting for differences in fixed effects of breed and season and their interaction as well as the random effect of animal.

The results of FEC analysis indicate that intensity of GIN infections was found to be low in Bonga and Horro sheep under CBBP (Table 2). The least square means (LSM) and standard errors (SE) of log transformed FEC during the dry season were 5.78 ± 0.08 and 3.82 ± 0.07, respectively, in Bonga and Horro CBBP. The corresponding FEC values during the rainy season were 5.05 ± 0.07 and 4.91 ± 0.06. On the other hand, high frequency of anthelmintics use was reported in sheep managed under CBBP communities as compared to non CBBP communities both at Bonga and Horro study area (Section **4.2**; Table 7). The low level of GIN infection can be ascribed to the high frequency of anthelmintic use which, in turn, likely is due to intervention of the breeding program in the studied communities. The analysis of Spearman’s correlation showed no association between FEC and FAMACHA scores in the study animals (Table 3).

The results of questionnaire survey (n = 240) complemented with Participatory Epidemiology (PE) study are presented under section **4.2** of the thesis**.** The data were used to assess the indigenous knowledge, practices and control options of GIN among CBBP and non CBBP communities of Bonga and Horro. The Cochran–Mantel–Haenszel $\chi 2$ test was used to determine associations between communities and frequencies of anthelmintic use for control of GIN of sheep. Differences between communities involved and not involved in CBBP for preferences of control methods of GIN in sheep were analyzed using Mann-Whitney U test.

The results of questionnaire and pairwise ranking demonstrated that diarrhea and bottle jaw are among the most important disease conditions of sheep of Bonga and Horro in CBBP (Table 4). These presumably are related to GIN (Vande Velde et al., 2018). Similar to livestock farmers in Mexico who consider bottle jaw, a disease by itself (Schillhorn van Veen, 1997), in our study, sheep farmers named diseases based on signs. The current heavy reliance on anthelmintic use by CBBP communities than non CBBP communities (Table 4) suggests that introduction of more sustainable control options for GIN of sheep is important in these communities. However, such farmers' positive attitude toward anthelmintics use, may be a barrier for possible uptake of sustainable control method, such as genetic selection in CBBP (Vande Velde et al., 2018). Moreover, CBBP farmers in Horro who rated anthelmintic as a sustainable option for control of GIN significantly higher than non CBBP farmers (Table 8), indicates that they lack awareness of anthelmintic resistance.

CBBP communities at both Horro and Bonga showed better understating on harmful effects and transmission of helminth parasites of sheep than non CBBP communities. Both CBBP and non CBBP farmers in Horro did not use ethnoveterinary medicinal plants for the control of GIN in sheep. However, in Bonga, farmers were accustomed to using the ethnoveterinary medicinal plants for the control of GIN in sheep. The most frequently used medicinal plant species in Bonga were *Ocimum lamiifolium*, *Nicotiana tabacum* and *Pycnostachys abyssinica* (Table 6).

Section **4.3** of the thesis presents inbreeding and population fine structure of Ethiopian sheep in CBBP. Single nucleotide polymorphism (SNP) genotype data (OvineHD BeadChip, n = 107), sampled from three CBBP villages (Boka, Buta and Shuta) were used for analysis of inbreeding levels of Ethiopian Bonga sheep. The inbreeding levels were measured based on the runs of homozygosity (ROH). The LSM ± SE of $F_{ROH}$ for the minimum length of ROH regions ($F_{ROH > 0.67\ Mb}$) were 0.068 ± 0.005, 0.060 ± 0.011 and 0.060 ± 0.007, respectively, in Boka, Buta and Shuta. The CBBP villages did not differ ($p > 0.05$) in the $F_{ROH}$ for all ROH length categories considered. The study showed low level of inbreeding in the Bonga CBBP. The analysis of inbreeding levels from ROH is becoming increasingly important in livestock species due to recent advances in SNP genotyping technology (Curik et al., 2014; Keller et al., 2011; McQuillan, et al., 2008). The advantages of valuating genomic inbreeding ($F_{ROH}$) over traditional (pedigree-based) inbreeding is that it is more accurate and potentially useful in animals lacking pedigree information, especially in developing countries (Peripolli et al., 2018; Keller et al., 2011; Keller et al., 2011; McQuillan, et al., 2008).

The NetView pipeline was used to reveal substructures of Bonga sheep at the CBBP villages. For this purpose, additional Ovine SNP50 BeadChip (n = 68) for structure analysis were used as a reference. The result showed absence of substructuring of populations in the Bonga CBBP.

Generally, the results from this study would help to develop nematodes control strategy and minimize inbreeding for sheep managed under community-based breeding schemes in Ethiopia. Thus, the following recommendations are given:

- FEC should be recorded rather than FAMACHA as a nematode resistance trait to be incorporated into the breeding goal of the CBBP of Bonga and Horro.

- The high anthelmintic use frequency in the CBBP should be reduced to minimize risk of anthelmintic drug resistance, possibly by integration with other alternatives for nematodes control, such as genetic selection of resistant sheep to GIN.
- While the levels of inbreeding in Bonga sheep in CBBP are currently low, management of inbreeding is important with the implementation of controlled breeding in CBBP. Exchange of breeding rams between grazing areas should be kept up.
- The absence of sub-structures of these sheep populations suggests that the breeding program may use rams across the CBBP villages.

## References


Abebe, R., Gebreyohannes, M., Mekuria, S., Abunna, F., Regassa, A., 2010. Gastrointestinal nematode infections in small ruminants under the traditional husbandry system during the dry season in southern Ethiopia. Trop. Anim. Health Prod. 42, 1111–1117. https://doi.org/10.1007/s11250-010-9532-3

Abegaz, S., Abebe, G., Awgichew, K., 2008. Sheep and goat production systems in Ethiopia. Sheep Goat Prod. Handb. Ethiop. Ethiop. Sheep Goat Product. Improv. Program ESGPIP 27–32.

Aga, T.S., Tolossa, Y.H., Terefe, G., 2013. Epidemiology of gastrointestinal nematodes of Horro sheep in Western Oromiya, Ethiopia. J. Vet. Med. Anim. Health 5(10), 296–304.

Alhusain, L., Hafez, A.M., 2018. Nonparametric approaches for population structure analysis. Hum. Genomics 12, 25.

Athanasiadou, S., Githiori, J., Kyriazakis, I., 2007. Medicinal plants for helminth parasite control: facts and fiction. Animal 1, 1392–1400.

Awas, T., Demissew, S., 2009. Ethnobotanical study of medicinal plants in Kafficho people, southwestern Ethiopia, in: Proceedings of the 16th International Conference of Ethiopian Studies. NTNU-Trykk Press, pp. 711–726.

Balmer, N., Torgerson, P., Hertzberg, H., 2015. Strategic control of gastrointestinal nematodes in grazing sheep with a long-acting moxidectin formulation. Small Rumin. Res. 126, 80–89. https://doi.org/10.1016/j.smallrumres.2015.03.012

Bath, G.F., van Wyk, J.A., 2009. The Five Point Check© for targeted selective treatment of internal parasites in small ruminants. Small Rumin. Res., Special Issue: Keynote Lectures of the 7th International Sheep Veterinary Congress 86, 6–13.

Besier, R.B., Kahn, L.P., Sargison, N.D., Van Wyk, J.A., 2016. Diagnosis, treatment and management of Haemonchus contortus in small ruminants, in: Advances in Parasitology. Elsevier, pp. 181–238.

Biffa, D., Jobre, Y., Chakka, H., 2006. Ovine helminthosis, a major health constraint to productivity of sheep in Ethiopia. Anim. Health Res. Rev. 7, 107–118. https://doi.org/10.1017/S1466252307001132

Blouin, S.F., Blouin, M., 1988. Inbreeding avoidance behaviors. Trends Ecol. Evol. 3, 230–233. https://doi.org/10.1016/0169-5347(88)90164-4

Burke, J.M., Kaplan, R.M., Miller, J.E., Terrill, T.H., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Vatta, A.F., 2007. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. Vet. Parasitol. 147, 89–95. https://doi.org/10.1016/j.vetpar.2007.03.033

Catley, A., Alders, R.G., Wood, J.L.N., 2012. Participatory epidemiology: Approaches, methods, experiences. Vet. J. 191, 151–160.

Catley, A., Okoth, S., Osman, J., Fison, T., Njiru, Z., Mwangi, J., Jones, B.A., Leyland, T.J., 2001. Participatory diagnosis of a chronic wasting disease in cattle in southern Sudan. Prev. Vet. Med. 51, 161–181.

Ceballos, F.C., Joshi, P.K., Clark, D.W., Ramsay, M., Wilson, J.F., 2018. Runs of homozygosity: windows into population history and trait architecture. Nat. Rev. Genet. 19, 220.

CSA (Central Statistical Agency), 2015. Agricultural Sample Survey 2014/15. Report on Livestock and Livestock Characteristics.

Curik, I., Ferenčaković, M., Sölkner, J., 2014. Inbreeding and runs of homozygosity: a possible solution to an old problem. Livest. Sci. 166, 26–34.

Datiko, J., Terefe, G., Bekele, J., 2013. Anthelmintic utilisation practices and prevalence of gastrointestinal helminth infections in sheep kept in the urban and peri-urban areas of Bishoftu Town. Trop. Anim. Health Prod. 45, 633–639.

Desta, H.G., 2015. Study on Prevalence of GI Nematodes in Indigenous Bonga Sheep Breed at Three Selected Agro Ecologies of Kaffa and Bench Maji Zones, Ethiopia. J. Biol. Agric. Healthc. 5, 77–85.

Dobon, B., Hassan, H.Y., Laayouni, H., Luisi, P., Ricaño-Ponce, I., Zhernakova, A., Wijmenga, C., Tahir, H., Comas, D., Netea, M.G., 2015. The genetics of East African populations: a Nilo-Saharan component in the African genetic landscape. Sci. Rep. 5, 9996.

Duguma, G., 2010a. Participatory definition of breeding objectives and implementation of community-based sheep breeding programs in Ethiopia. PhD Thesis, University of Natural Resources and Life Sciences, Vienna, Austria.

Duguma, G., 2010b. Participatory definition of breeding objectives and implementation of community-based sheep breeding programs in Ethiopia (PhD Thesis). PhD Thesis, University of Natural Resources and Life Sciences, Vienna, Austria.

Edea, Z., 2008. Characterization of Bonga and Horro indigenous sheep breeds of smallholders for designing community based breeding strategies in Ethiopia (PhD Thesis). Haramaya University.

Edea, Z., Dessie, T., Dadi, H., Do, K.-T., Kim, K.-S., 2017. Genetic diversity and population structure of Ethiopian sheep populations revealed by high-density SNP markers. Front. Genet. 8, 218.

Ferenčaković, M., Hamzić, E., Gredler, B., Solberg, T.R., Klemetsdal, G., Curik, I., Sölkner, J., 2013. Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. J. Anim. Breed. Genet. 130, 286–293.

Galal, E.S.E., 1983. Sheep germplasm of Ethiopia. Animal Genetic Resources Information (No. 1/83). FAO, Rome.

Gauly, M., Erhardt, G., 2001. Genetic resistance to gastrointestinal nematode parasites in Rhön sheep following natural infection. Vet. Parasitol. 102, 253–259. https://doi.org/10.1016/S0304-4017(01)00530-1

Getachew, T., Dorchies, P., Jacquiet, P., 2007. Trends and challenges in the effective and sustainable control of Haemonchus contortus infection in sheep. Review. Parasite Paris Fr. 14, 3–14. https://doi.org/10.1051/parasite/2007141003

Githiori, J.B., Athanasiadou, S., Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Vet. Parasitol. 139, 308–320.

Gizaw, S., 2010. Sheep and goat production and marketing systems in Ethiopia: Characteristics and strategies for improvement. ILRI (aka ILCA and ILRAD).

Gizaw, S., Arendonk, J.A.M.V., Komen, H., Windig, J.J., Hanotte, O., 2007. Population structure, genetic variation and morphological diversity in indigenous sheep of Ethiopia. Anim. Genet. 38, 621–628. https://doi.org/10.1111/j.1365-2052.2007.01659.x

Gizaw, S., Getachew, T., Edea, Z., Mirkena, T., Duguma, G., Tibbo, M., Rischkowsky, B., Mwai, O., Dessie, T., Wurzinger, M., Solkner, J., Haile, A., 2013. Characterization of indigenous breeding strategies of the sheep farming communities of Ethiopia: A basis for designing community-based breeding programs. ICARDA Work. Pap. Aleppo Syr. 49.

Grilz-Seger, G., Mesarič, M., Cotman, M., Neuditschko, M., Druml, T., Brem, G., 2018. Runs of homozygosity and population history of three horse breeds with small population size. J. Equine Vet. Sci. 71, 27–34.

Haile, A., Gashaw, A., Tolemariam, T., Tibbo, M., 2010. Epidemiology of nematode parasites of sheep around Jimma, southwestern Ethiopia. Trop. Anim. Health Prod. 42, 825–831. https://doi.org/10.1007/s11250-009-9492-7

Heckendorn, F., Bieber, A., Werne, S., Saratsis, A., Maurer, V., Stricker, C., 2017. The genetic basis for the selection of dairy goats with enhanced resistance to gastrointestinal nematodes. Parasite 24, 32. https://doi.org/10.1051/parasite/2017033

IBM SPSS Statistics 20, 2011. IBM SPSS statistics base 20. Chicago, IL: SPSS Inc.

Idris, A., Moors, E., Sohnrey, B., Gauly, M., 2012. Gastrointestinal nematode infections in German sheep. Parasitol. Res. 110, 1453–1459.

Iqbal, Z., Lateef, M., Jabbar, A., Ghayur, M.N., Gilani, A.H., 2006. In vitro and In vivo anthelmintic activity of Nicotiana tabacum L. leaves against gastrointestinal nematodes of sheep. Phytother. Res. 20, 46–48.

Johnston, S.E., Bérénos, C., Slate, J., Pemberton, J.M., 2016. Conserved Genetic Architecture Underlying Individual Recombination Rate Variation in a Wild Population of Soay Sheep (Ovis aries). Genetics 203, 583–598. https://doi.org/10.1534/genetics.115.185553

Kaplan, R., Burke, J., Terrill, T., Miller, J., Getz, W.R., Mobini, S., Valencia, E., Williams, M., Williamson, L., Larsen, M., Vatta, A., 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol. 123, 105–120. https://doi.org/10.1016/j.vetpar.2004.06.005

Keller, M.C., Visscher, P.M., Goddard, M.E., 2011. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. Genetics 189, 237–249.

Khajuria, J.K., Katoch, R., Yadav, A., Godara, R., Gupta, S.K., Singh, A., 2013. Seasonal prevalence of gastrointestinal helminths in sheep and goats of middle agro-climatic zone of Jammu province. J. Parasit. Dis. Off. Organ Indian Soc. Parasitol. 37, 21–25. https://doi.org/10.1007/s12639-012-0122-3

Khan, M.N., Sajid, M.S., Khan, M.K., Iqbal, Z., Hussain, A., 2010. Gastrointestinal helminthiasis: prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. Parasitol. Res. 107, 787–794. https://doi.org/10.1007/s00436-010-1931-x

Kijas, J.W., Lenstra, J.A., Hayes, B., Boitard, S., Neto, L.R.P., San Cristobal, M., Servin, B., McCulloch, R., Whan, V., Gietzen, K., 2012. Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. PLoS Biol. 10, e1001258.

Kijas, J.W., Townley, D., Dalrymple, B.P., Heaton, M.P., Maddox, J.F., McGrath, A., Wilson, P., Ingersoll, R.G., McCulloch, R., McWilliam, S., 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PloS One 4, e4668.

Koopmann, R., Holst, C., Epe, C., 2006. Experiences with the FAMACHA-Eye-Colour-Chart for identifying sheep and goats for targeted anthelmintic treatment. Berl. Munch. Tierarztl. Wochenschr. 119, 436–442.

Kukučková, V., Moravčíková, N., Ferenčaković, M., Simčič, M., Mészáros, G., Sölkner, J., Trakovická, A., Kadlečík, O., Curik, I., Kasarda, R., 2017. Genomic characterization of Pinzgau cattle: genetic conservation and breeding perspectives. Conserv. Genet. 18, 893–910.

Leta, S., Mesele, F., 2014. Spatial analysis of cattle and shoat population in Ethiopia: growth trend, distribution and market access. SpringerPlus 3, 310. https://doi.org/10.1186/2193-1801-3-310

Malan, F.S., Wessels, C.D., Van Wyk, J.A., 2001. Clinical evaluation of anaemia in sheep: early trials.

Marras, G., Gaspa, G., Sorbolini, S., Dimauro, C., Ajmone-Marsan, P., Valentini, A., Williams, J.L., Macciotta, N.P., 2015. Analysis of runs of homozygosity and their relationship with inbreeding in five cattle breeds farmed in Italy. Anim. Genet. 46, 110–121.

Mastrangelo, S., Ciani, E., Sardina, M.T., Sottile, G., Pilla, F., Portolano, B., 2018. Runs of homozygosity reveal genome-wide autozygosity in Italian sheep breeds. Anim. Genet. 49, 71–81. https://doi.org/10.1111/age.12634

Mavrot, F., Hertzberg, H., Torgerson, P., 2015. Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. Parasit. Vectors 8, 557. https://doi.org/10.1186/s13071-015-1164-z

McKay, S.D., Schnabel, R.D., Murdoch, B.M., Matukumalli, L.K., Aerts, J., Coppieters, W., Crews, D., Neto, E.D., Gill, C.A., Gao, C., 2008. An assessment of population structure in eight breeds of cattle using a whole genome SNP panel. BMC Genet. 9, 37.

McQuillan, R., Leutenegger, A.-L., Abdel-Rahman, R., Franklin, C.S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., 2008. Runs of homozygosity in European populations. Am. J. Hum. Genet. 83, 359–372.

Meijer, S.S., Catacutan, D., Ajayi, O.C., Sileshi, G.W., Nieuwenhuis, M., 2015. The role of knowledge, attitudes and perceptions in the uptake of agricultural and agroforestry innovations among smallholder farmers in sub-Saharan Africa. Int. J. Agric. Sustain. 13, 40–54.

Miller, J.E., Horohov, D.W., 2006. Immunological aspects of nematode parasite control in sheep. J. Anim. Sci. 84, E124–E132. https://doi.org/10.2527/2006.8413_supplE124x

Mirkena, T., Duguma, G., Willam, A., Wurzinger, M., Haile, A., Rischkowsky, B., Okeyo, A.M., Tibbo, M., Solkner, J., 2012. Community-based alternative breeding plans for indigenous sheep breeds in four agro-ecological zones of Ethiopia. J. Anim. Breed. Genet. 129, 244–253. https://doi.org/10.1111/j.1439-0388.2011.00970.x

Moors, E., Gauly, M., 2009. Is the FAMACHA© chart suitable for every breed? Correlations between FAMACHA© scores and different traits of mucosa colour in naturally parasite infected sheep breeds. Vet. Parasitol. 166, 108–111. https://doi.org/10.1016/j.vetpar.2009.07.040

Morgan, E.R., Hosking, B.C., Burston, S., Carder, K.M., Hyslop, A.C., Pritchard, L.J., Whitmarsh, A.K., Coles, G.C., 2012. A survey of helminth control practices on sheep farms in Great Britain and Ireland. Vet. J. 192, 390–397.

Mueller, J.P., Rischkowsky, B., Haile, A., Philipsson, J., Mwai, O., Besbes, B., Valle Zárate, A., Tibbo, M., Mirkena, T., Duguma, G., 2015. Community-based livestock breeding programmes: essentials and examples. J. Anim. Breed. Genet. 132, 155–168.

Nandolo, W., Mészáros, G., Banda, L.J., Gondwe, T.N., Lamuno, D., Mulindwa, H.A., Nakimbugwe, H.N., Wurzinger, M., Utsunomiya, Y.T., Woodward-Greene, M.J., Liu, M., Liu, G., Van Tassell, C.P., Curik, I., Rosen, B.D., Sölkner, J., 2019. Timing and Extent of Inbreeding in African Goats. Front. Genet. 10. https://doi.org/10.3389/fgene.2019.00537

Neuditschko, M., Khatkar, M.S., Raadsma, H.W., 2012. NetView: a high-definition network-visualization approach to detect fine-scale population structures from genome-wide patterns of variation. PloS One 7, e48375.

Norberg, E., Sørensen, A.C., 2007. Inbreeding trend and inbreeding depression in the Danish populations of Texel, Shropshire, and Oxford Down. J. Anim. Sci. 85, 299–304.

Notter, D.R., Burke, J.M., Miller, J.E., Morgan, J.L.M., 2017a. Factors affecting fecal egg counts in periparturient Katahdin ewes and their lambs. J. Anim. Sci. 95, 103–112. https://doi.org/10.2527/jas.2016.0955

Notter, D.R., Burke, J.M., Miller, J.E., Morgan, J.L.M., 2017b. Association between FAMACHA scores and fecal egg counts in Katahdin lambs. J. Anim. Sci. 95, 1118–1123. https://doi.org/10.2527/jas.2016.1248

Nouri, F., Nourollahi-Fard, S.R., Foroodi, H.R., Sharifi, H., 2016. In vitro anthelmintic effect of Tobacco (Nicotiana tabacum) extract on parasitic nematode, Marshallagia marshalli. J. Parasit. Dis. 40, 643–647.

Nwosu, C.O., Madu, P.P., Richards, W.S., 2007. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. Vet. Parasitol. 144, 118–124. https://doi.org/10.1016/j.vetpar.2006.09.004

Peripolli, E., Munari, D.P., Silva, M., Lima, A.L.F., Irgang, R., Baldi, F., 2017. Runs of homozygosity: current knowledge and applications in livestock. Anim. Genet. 48, 255–271.

Peripolli, E., Stafuzza, N.B., Munari, D.P., Lima, A.L.F., Irgang, R., Machado, M.A., Panetto, J.C. do C., Ventura, R.V., Baldi, F., da Silva, M.V.G.B., 2018. Assessment of runs of homozygosity islands and estimates of genomic inbreeding in Gyr (Bos indicus) dairy cattle. BMC Genomics 19, 34. https://doi.org/10.1186/s12864-017-4365-3

Petit, M., Astruc, J.-M., Sarry, J., Drouilhet, L., Fabre, S., Moreno, C.R., Servin, B., 2017. Variation in Recombination Rate and Its Genetic Determinism in Sheep Populations. Genetics 207, 767–784. https://doi.org/10.1534/genetics.117.300123

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., Bakker, P.I.W. de, Daly, M.J., Sham, P.C., 2007. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am. J. Hum. Genet. 81, 559–575. https://doi.org/10.1086/519795

Purfield, D.C., Berry, D.P., McParland, S., Bradley, D.G., 2012. Runs of homozygosity and population history in cattle. BMC Genet. 13, 70.

R Core Team, 2017. R: A Language and Environment for Statistical Computing.

Regassa, F., Teshale, S., Dhuguma, R., Kiros, Y., 2006. Epidemiology of gastrointestinal parasites of ruminants in Western Oromia, Ethiopia. Int. J. Appl. Res. Vet. Med. 4, 51–57.

Riley, D.G., Van Wyk, J.A., 2009. Genetic parameters for FAMACHA© score and related traits for host resistance/resilience and production at differing severities of worm challenge in a Merino flock in South Africa. Vet. Parasitol. 164, 44–52.

Roeber, F., Jex, A.R., Gasser, R.B., 2013a. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective. Parasit. Vectors 6, 153. https://doi.org/10.1186/1756-3305-6-153

Roeber, F., Jex, A.R., Gasser, R.B., 2013b. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective. Parasit. Vectors 6, 153.

Rosenberg, N.A., Pritchard, J.K., Weber, J.L., Cann, H.M., Kidd, K.K., Zhivotovsky, L.A., Feldman, M.W., 2002. Genetic structure of human populations. science 298, 2381–2385.

Russell, T., 1997. Pair wise ranking made easy. PLA Notes CD - ROM 1988 – 2001 25–26.

SAS Institute Inc., 2012. SAS Institute Inc Cary, NC, USA.

Scantlebury, C.E., Peachey, L., Hodgkinson, J., Matthews, J.B., Trawford, A., Mulugeta, G., Tefera, G., Pinchbeck, G.L., 2013. Participatory study of medicinal plants used in the control of gastrointestinal parasites in donkeys in Eastern Shewa and Arsi zones of Oromia region, Ethiopia. BMC Vet. Res. 9, 179. https://doi.org/10.1186/1746-6148-9-179

Scheuerle, M., Mahling, M., Muntwyler, J., Pfister, K., 2010. The accuracy of the FAMACHA©-method in detecting anaemia and haemonchosis in goat flocks in Switzerland under field conditions. Vet. Parasitol. 170, 71–77. https://doi.org/10.1016/j.vetpar.2010.01.035

Schillhorn van Veen, T.W., 1997. Sense or nonsense? Traditional methods of animal parasitic disease control. Vet. Parasitol., Plenary Papers of the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology 71, 177–194.

Stear, M.J., 2010. Breeding for resistance to nematode infections., in: Bishop, S.C., Axford, R.F.E., Nicholas, F.W., Owen, J.B. (Eds.), Breeding for Disease Resistance in Farm Animals. CABI, Wallingford, pp. 279–294.

Stear, M.J., Doligalska, M., Donskow-Schmelter, K., 2007. Alternatives to anthelmintics for the control of nematodes in livestock. Parasitology 134, 139–151.

Steinig, E.J., Neuditschko, M., Khatkar, M.S., Raadsma, H.W., Zenger, K.R., 2016. netview p: a network visualization tool to unravel complex population structure using genome-wide SNP s. Mol. Ecol. Resour. 16, 216–227.

Sungirai, M., Moyo, D.Z., De Clercq, P., Madder, M., 2016. Communal farmers’ perceptions of tick-borne diseases affecting cattle and investigation of tick control methods practiced in Zimbabwe. Ticks Tick-Borne Dis. 7, 1–9. https://doi.org/10.1016/j.ttbdis.2015.07.015

Taylor, M.A., 2010. Parasitological examinations in sheep health management. Small Rumin. Res. 92, 120–125. https://doi.org/10.1016/j.smallrumres.2010.04.012

Thrusfield, M., 2005. Veterinary epidemiology, 3rd ed. Blackwell publishing, Oxford.

Torgerson, P., Claxton, J., 1999. Epidemiology and Control, in: Fasciollosis. CABI publishing, Dublin City University, Republic of Ireland, pp. 113–150.

Traoré, A., Notter, D.R., Soudre, A., Kaboré, A., Álvarez, I., Fernández, I., Sanou, M., Shamshuddin, M., Periasamy, K., Tamboura, H.H., Goyache, F., 2017. Resistance to gastrointestinal parasite infection in Djallonké sheep. animal 11, 1354–1362. https://doi.org/10.1017/S1751731116002640

Urquhart, G.M., Armour, J., Duncan, J.L., Jennings, F.W., Dunn, A.M., 1996. Veterinary Parasitology, 2nd ed. ed. Wiley-Blackwell, Oxford, UK.

Vande Velde, F., Charlier, J., Claerebout, E., 2018. Farmer behavior and gastrointestinal nematodes in ruminant livestock–uptake of sustainable control approaches. Front. Vet. Sci. 5, 255.

Vilela, V.L.R., Feitosa, T.F., Linhares, E.F., Athayde, A.C.R., Molento, M.B., Azevedo, S.S., 2012. FAMACHA© method as an auxiliary strategy in the control of gastrointestinal helminthiasis of dairy goats under semiarid conditions of Northeastern Brazil. Vet. Parasitol. 190, 281–284. https://doi.org/10.1016/j.vetpar.2012.05.024

Waller, P.J., 2006. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Anim. Feed Sci. Technol., Feed and Animal Health 126, 277–289.

Waller, P.J., Thamsborg, S.M., 2004. Nematode control in ‘green’ruminant production systems. Trends Parasitol. 20, 493–497.

Wyk, J.A.V., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529. https://doi.org/10.1051/vetres:2002036

Zvinorova, P.I., Halimani, T.E., Muchadeyi, F.C., Katsande, S., Gusha, J., Dzama, K., 2017. Management and control of gastrointestinal nematodes in communal goat farms in Zimbabwe. Trop. Anim. Health Prod. 49, 361–367.

# Annexes

## Annex 1. Questionnaire

**Objective:** to assess local knowledge about, current control practices and preferences of gastrointestinal nematode (GIN) parasites of sheep of Bonga and Horro, Ethiopia

☐ **Consent**: Put (√) mark in the box to confirm the consent that the interviewee is informed about the purpose of gathering this information (as a part of PhD thesis work of Mr. Solomon Shiferaw Tufa, the findings will contribute to strengthen the capacity of the CBBP in regards to genetic improvement of GIN of sheep).

### I. General characteristics of respondents

Study area (1 = Horro, 2 = Bonga); Study site (community) _________________;
Respondent's name (HH id ) ______________________________

### II. General health problems of sheep in the study areas, ethnoveterinary knowledge and practices

1) Please list the major health problems (diseases/parasites) of sheep on your farm. Also, rank them in their order of importance.

| **Local name** | **Signs** observed | **Sc. Name** (to be interpreted later) | **Rank*** |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

*1 = most important, $X_n$ = least important

2) Please see color pictures and fill the table below (whether or not you have ever seen?, when?, where the location in the sheep?, what local names for each, and perceived disease/sign caused by each worm?) [show color pictures of representative (adult) worm parasites of sheep]

| **Worm type shown** | **seen** | | | **Worms'** | | |
|---|---|---|---|---|---|---|
| | **Yes** | **No** | ***when** | ****location** | **Local name** | *****Perceived disease/signs** |
| a) lung worm | □ | □ | □ | | | |
| b) liver fluke | □ | □ | □ | | | |
| c) rumen fluke | □ | □ | □ | | | |
| d) Haemonchus spp | □ | □ | □ | | | |
| e) tape worm | □ | □ | □ | | | |
| f) Trichuris spp | □ | □ | □ | | | |

*1 = slaughtered for consumption, 2 = died of unknown disease, 3 = 1&2
**1 = liver, 2 = rumen, 3 = abomasums, 4 = small intestine, 5 = large intestine, 6 = trachea/lung, 7 = others
***1 = coughing, 2 = diarrhea, 3 = bottle jaw, 4 = weight loss, d) anaemia, e = death (sudden)

3) Please also indicate if you have ever seen any type of the above worms in faeces of sheep.
………………………………………………………………………………………………………
……………………………………..

4) Do you agree or disagree if the worms are grouped and named as below? [N.B. see pictures]

| **worms shown as grouped** | **Worms' group name** | **Agree** | **Disagree** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| All (a-f) | *gastro-intestinal (GI) worms | □ | □ |
| b & e | flat worms | □ | □ |
| a,d & f | *round worms | □ | □ |
| a, d & f | *GI round worms | □ | □ |

*context explained before questioning (after obtaining their responses all are told to understand this context for later use)

5) Which type of [these] sheep worms are more serious problem for your flock? [tick only one item]

□1 GI round worms □2 liver flukes □3 don't know

6) Do you know how the worms get entry into the sheep stomach/intestine? [tick only one item]

□1 inborn □2 acquired (eg., ingested with grass) □3 don't know

7) Do you agree or disagree with each of the following statements?

| There are (stomach/intestinal) worms that: | | **Yes** | **No** |
|---|---|---|---|
| 7.1) | are useful to sheep | □ | □ |
| 7.2) | cause weight loss/slow growth rate in lambs | □ | □ |
| 7.3) | cause diarrhea | □ | □ |
| 7.4) | suck blood | □ | □ |
| 7.5) | may cause death | □ | □ |

8) Have you ever received training on internal parasites /GI round worms and their control?

□1 Yes □2 No

8.1) If yes, who provided you the training? [tick one or more relevant items]

□1 Gov vet service □2) private vet practitioners □3 research centers □4 NGO

9) Which of the following methods of control of GI round worms do you use for your flock?

| **Technology options for GI round worms control** | | **Yes** | **No** |
|---|---|---|---|
| 9.1) | traditional medicines (ethnoveterinary remedies) | □ | □ |
| 9.2) | anthelimntic | □ | □ |
| 9.3) | selective breeding | □ | □ |
| 9.4) | nutritional supplementation | □ | □ |
| 9.5) | grazing management | □ | □ |

10) If you use traditional methods, who treats your sheep?

□1 yourself □2 traditional healers □3 others (specify)…………………..

10.1) if yourself, from where do you learn the knowledge?

□1 parents □2 traditional healer □3 development programs □4 others (specify) ……

| 10.2) if you use plant sources, what parts do you use)? | Yes | No | Season (1 = wet, 2 = dry, 3 = both) | Plant's name (local) |
|---|---|---|---|---|
| roots | □ | □ | □ | |
| leaves | □ | □ | □ | |
| barks | □ | □ | □ | |
| Others (specify) | □ | □ | □ | |

10.3) How do you administer the (traditional) medicinal plant? [tick one or more relevant]

□1 bolusing □2 drenching □3 mixed with feed □4 others (specify)…………

**III. Communities' knowledge on anthelmintic usage** (types, frequency, aspects of epidemiology of GIN of sheep—age, sex, and season, etc.)

| 1)How often, in the last 1 year, you did use (the following anthelmintic*) for your sheep? | never | once | twice | more (specify) | anthelmintic's local name |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Albendazole* | | | | | |
| Tetramisole* | | | | | |
| Tetramisole + Levamisole* | | | | | |
| Ivermectin* | | | | | |
| Triclabendazole (Fasinex) | | | | | |

* shown real anthelmintic type

2) Why did you prefer to using a particular anthelmintic type (see above #1)?

| **Reasons** | **Rank** (top 3) |
|---|---|
| worm type | |
| Color (………..……………….please indicate the chosen color) | |
| Cost (low) | |
| Availability (local market) | |
| Ease of administration (oral, injection, etc) | |
| Efficacy/effectiveness (cure, good, etc) | |
| Veterinarians prescription (or as incentive given by research/project ….) | |

3) If "worm type" was ranked 1-3 (see above), which specific anthelmintic type do you use to which specific worm groups?

| **anthelmintic type** | **Worm type/groups** |
|---|---|
| | |
| | |
| | |

4) How is the trend (in frequency) of anthelmintic usage in your sheep in the last 5 years? [tick one]

☐1 increased ☐2 decreased ☐3 no change

| | use | | If yes, how was the improvement? | | |
|---|---|---|---|---|---|
| 5) Do (did) you use anthelmintic, in your sheep flock, for; | Yes | No | highly improved | improved | not improved |
| 5.1) Sick animal (coughing, diarrhea, bottle jaw) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 5.2) apparently healthy (but poor body condition) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 5.3) Healthy (and good body condition) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 5.4) others (please specify……………………) | ☐ | ☐ | ☐ | ☐ | ☐ |

6) Do you get advisory services on anthelmintic usage for GI worms of sheep? [tick 1 or more relevant items]

☐1 GOV vet service ☐2 private vet practitioners ☐3 research centers ☐4 NGO ☐5 none

| 7) Do (did) you use anthelmintic, in your sheep flock, for; | | Use | | Season (1 = wet, 2 = dry, 3 = both) | Reasons |
|---|---|---|---|---|---|
| Age | Sex (& others) | Yes | No | | |
| lambs (0-3 months) | male | ☐ | ☐ | ☐ | |
| | female | ☐ | ☐ | ☐ | |
| lambs (4-6 months) | male | ☐ | ☐ | ☐ | |
| | female | ☐ | ☐ | ☐ | |

| | | | | | |
|---|---|---|---|---|---|
| lambs (>6-12 months) | male | ◻ | ◻ | ◻ | |
| | female | ◻ | ◻ | ◻ | |
| yearlings (1-1.5 year) | male | ◻ | ◻ | ◻ | |
| | female | ◻ | ◻ | ◻ | |
| Adults (> 1.5 year) | lactating ewes | ◻ | ◻ | ◻ | |
| | pregnant ewes | ◻ | ◻ | ◻ | |
| | non-pregnant ewes | ◻ | ◻ | ◻ | |
| | breeding ram(s) | ◻ | ◻ | ◻ | |
| | fattening ram(s) | ◻ | ◻ | ◻ | |

8) Where from do you get/buy anthelmintic for your sheep? [tick one or more relevant items]
☐1 open market/non-vet drug shop ☐2 (human) drug store ☐3 private vet drug shop
☐4 government vet clinic ☐5 others (e.g., development/research projects)
9) Over the last year, how much did you expend for buying anthelmintic for your sheep flock?
…………………………………………………Birr (average estimated cost for anthelmintic/year).

**IV. knowledge, attitudes and preferences of alternative control options to anthelmintic for GIN among sheep breeding communities**

1) In your opinion, what do you think of the anthelmintic treatment of all sheep in your flock?
☐1 very bad ☐2 bad ☐3 not sure ☐4 good ☐5 very good

| 2) | Over the last 1 year, how would you judge a ewe in your flock for that you didn't give any anthelmintic (skip if not relevant) | **Body condition** (1 = very poor to 5 = very good) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 2.1 | How was her own body condition? | ◻ | ◻ | ◻ | ◻ | ◻ |
| 2.2 | How was her previous lambs' condition? | ◻ | ◻ | ◻ | ◻ | ◻ |
| 2.3 | What do you think would be her future lambs' condition? | ◻ | ◻ | ◻ | ◻ | ◻ |

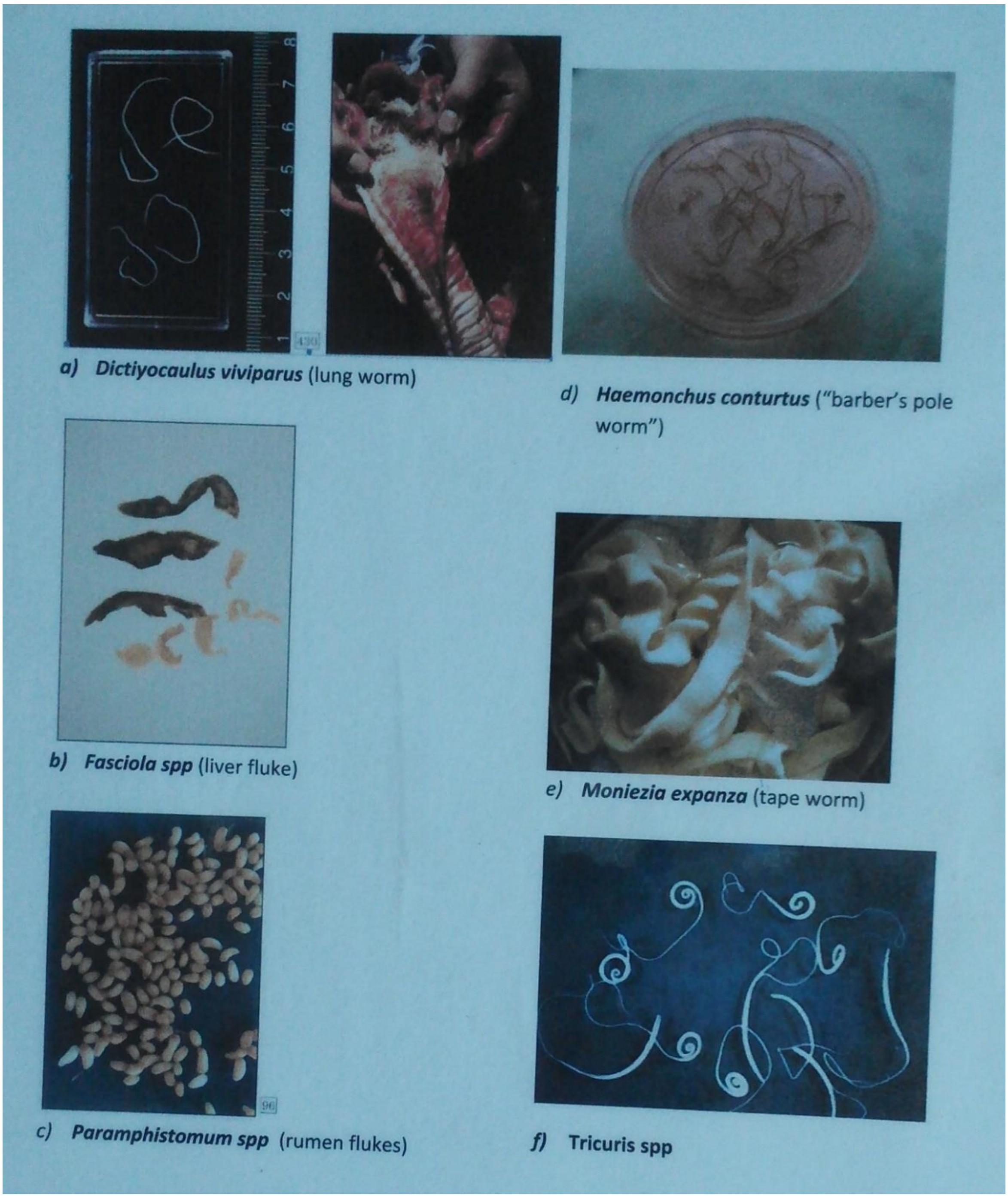
3) Do you agree or disagree with the following statements? (please tick one box per question)
3.1) In my flock there are some ewes that need less anthelmintic drugs than others due to inheritance from their parents?
☐1 strongly disagree ☐2 disagree ☐3 not sure ☐4 agree ☐5 strongly agree
3.2) A lamb from a ewe that requires less anthelmintic treatment per year also requires less.
☐1 strongly disagree ☐2 disagree ☐3 not sure ☐4 agree ☐5 strongly agree
3.3) There is no sheep in my flock that resist harmful effects of GI parasites.
☐1 strongly disagree ☐2 disagree ☐3 not sure ☐4 agree ☐5 strongly agree

4) Please rate your preference of technology options for GIN control in your sheep. [Rate using a five point Likert -type scales as indicated below]

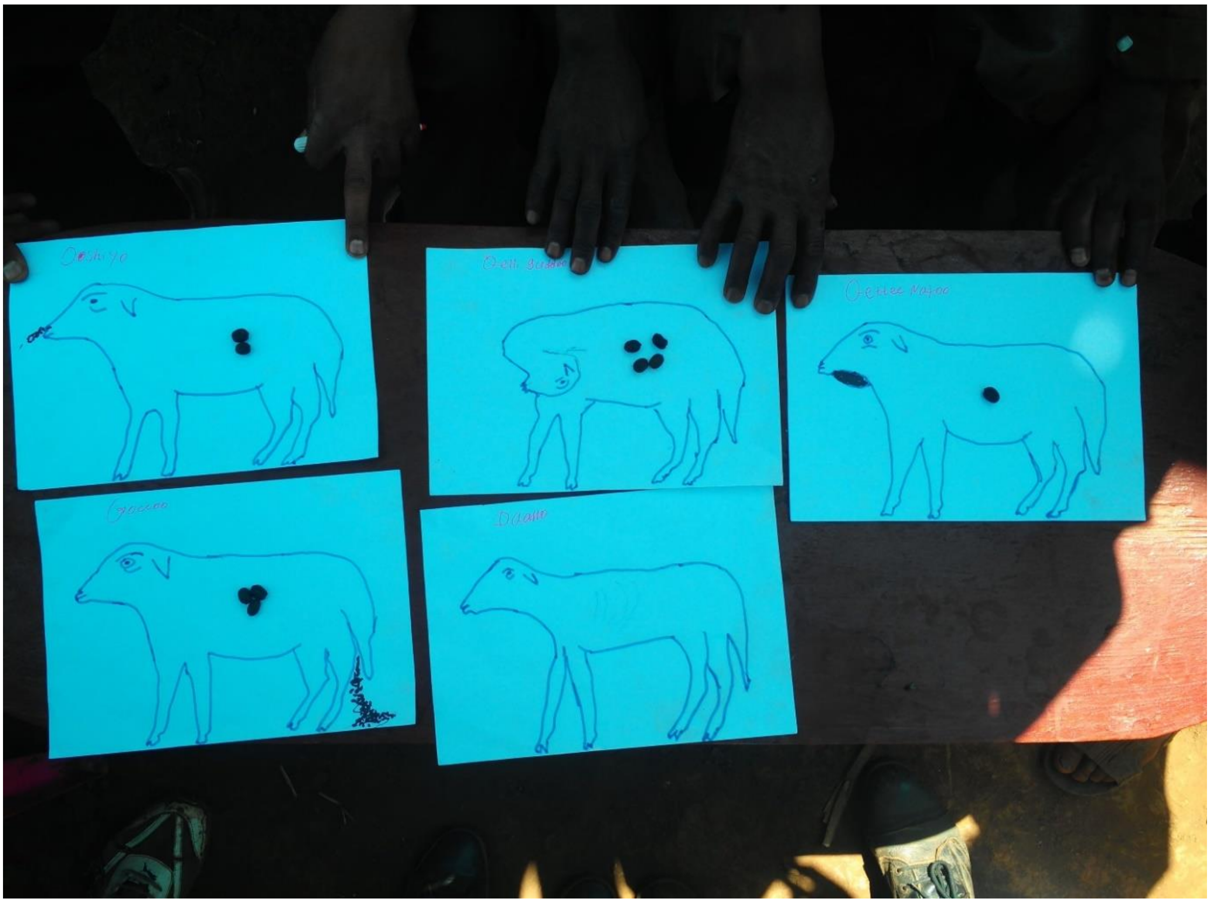
| | | **availability** (1 = hardly available to 5 = easily available) | | | | | |
|---|---|---|---|---|---|---|---|
| 4.2) | How available is each of the following methods for control of GIN parasites in your flock? | 1 | 2 | 3 | 4 | 5 | |
| a | ethnoveterinary remedies | ◻ | ◻ | ◻ | ◻ | ◻ | |
| b | anthelimntic | ◻ | ◻ | ◻ | ◻ | ◻ | |
| c | selective breeding | ◻ | ◻ | ◻ | ◻ | ◻ | |
| d | nutritional supplementation | ◻ | ◻ | ◻ | ◻ | ◻ | |
| | | **cost** (1 = very expensive to 5 = very cheap | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4.3) | How costly is each of the following methods for control of GIN parasites in your flock? | 1 | 2 | 3 | 4 | 5 | |
| a | ethnoveterinary remedies | □ | □ | □ | □ | □ | |
| b | anthelimntic | □ | □ | □ | □ | □ | |
| c | selective breeding | □ | □ | □ | □ | □ | |
| d | nutritional supplementation | □ | □ | □ | □ | □ | |
| | | **effectiveness** (1 = not effective to 5 = very effective | | | | | |
| 4.4) | How effective is each of the following methods for control of GIN parasites in your flock? | 1 | 2 | 3 | 4 | 5 | |
| a | ethnoveterinary remedies | □ | □ | □ | □ | □ | |
| b | anthelimntic | □ | □ | □ | □ | □ | |
| c | selective breeding | □ | □ | □ | □ | □ | |
| d | nutritional supplementation | □ | □ | □ | □ | □ | |
| | | **ease of implementation** (1 = very complex to 5 = very simple) | | | | | |
| 4.5) | How easy is implementation of each of the following methods for control of GIN parasites in your flock? | 1 | 2 | 3 | 4 | 5 | |
| a | ethnoveterinary remedies | □ | □ | □ | □ | □ | |
| b | anthelimntic | □ | □ | □ | □ | □ | |
| c | selective breeding | □ | □ | □ | □ | □ | |
| d | nutritional supplementation | □ | □ | □ | □ | □ | |
| | | **sustainability** (1 = not sustainable to 5 = very sustainable | | | | | |
| 4.6) | How sustanable is each of the following methods for control of GIN parasites in your flock? | 1 | 2 | 3 | 4 | 5 | |
| a | ethnoveterinary remedies | □ | □ | □ | □ | □ | |
| b | anthelimntic | □ | □ | □ | □ | □ | |
| c | selective breeding | □ | □ | □ | □ | □ | |
| d | nutritional supplementation | □ | □ | □ | □ | □ | |

# Annex 2. Supplementary materials (questionnaire and PE)




**Supplementary material 1)** Color pictures of six helminth parasites of sheep, shown on a laminated A4 paper during interviewing. These represented nematodes (a, d and f); trematodes (b and c); and the cestode (e).



**Supplementary material 2a)** A sample pairwise ranking undertook in Bonga community-based breeding program (CBBP) by women group. The bigger the pile of seeds for a disease condition, the more important that disease condition in the CBBP sheep.

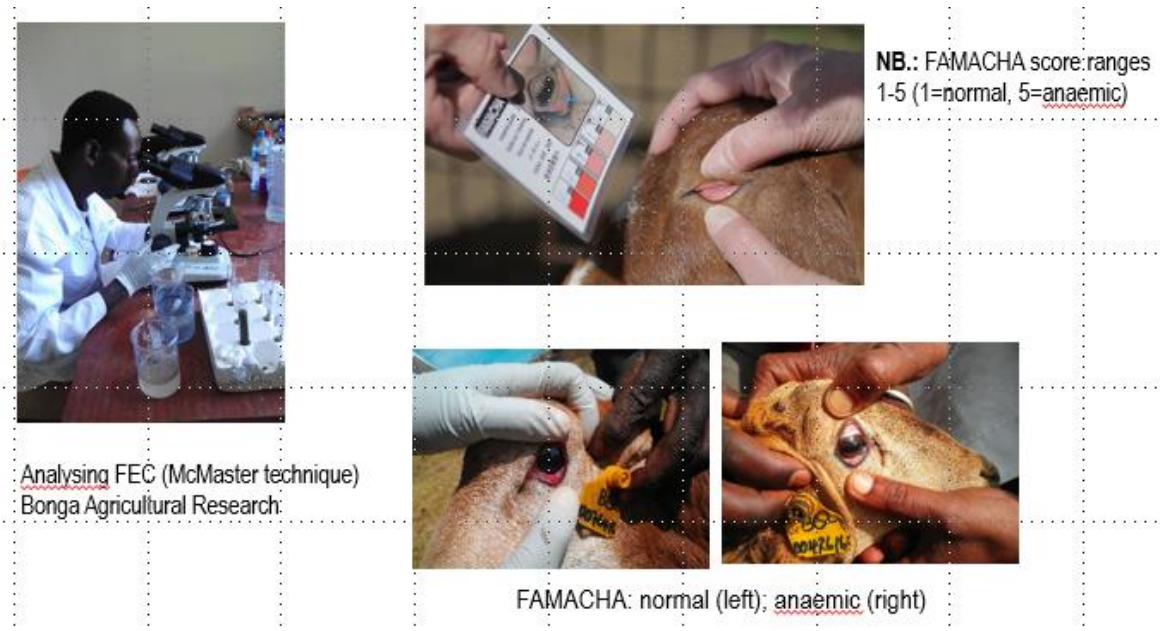
**Supplementary material 2b)** A sample pairwise ranking undertook in Horro CBBP by men group. The bigger the pile of seeds for a disease condition, the more important that disease condition in the CBBP sheep.

**Supplementary material 3a)** sample matrix scoring shown by drawings representing five major disease conditions of sheep (y-axis) and four GIN control methods in sheep (x-axis); anthelmintic, ethnoveterinary and nutritional supplementation represented by real objects, and selective breeding represented by two contrasting drawings of sheep for worm burden in stomach (low vs. high). NB.: Scores were not assigned (matrix before completion).


**Supplementary material 3b)** A sample matrix scoring after completed in Bonga CBBP by women participants, and the result being interviewed using a semi-structured interview (SSI).

# Annex 3. Selected pictures (field and lab studies)

**Annex 3.1** FEC and FAMACHA sampling during two seasons; wet (top) and dry (bottom) from two breeds/locations of CBBP, Horro (left) and Bonga (right)




**Annex 3.2** FEC and FAMACHA recording activities at Bonga and Horro

**Annex 3.3** rectal fresh faeces sample collection in Bonga

**Annex 3.4** Conducting face-to-face interviews (questionnaire survey)

**Annex 3.5** Different ethnoveterinary medicinal plants used by CBBP communities in Bonga

**Annex 3.6** Damo (*Ocimum lamiifolium*), one of ethnoveterinary medicinal plants used by CBBP communities in Bonga.