Original Research Article

Reduced albendazole efficacy in goats naturally infected with strongyle nematodes in Dagrase area, South Darfur State, Sudan

Abstract

Introduction: Understanding the extend of benzimidazole resistance is a core step to adopt the correct strategies for control. In Sudan, benzimidazole resistance was recently reported in cattle and goats in South Darfur. This study aimed to collect additional data regarding albendazole efficacy.

Methods: In the rainy season, 100 goats, male and female of different age groups, were screened for the presence of gastrointestinal helminth infections in Dagrase, South Darfur State, Sudan. Goats shedding >150 nematode eggs per gram faeces were selected for *in vivo* trial and grouped into control (*n*=15) and a treated group (*n*=70), that were individually received an oral dose of 10 mg/kg body weight albendazole. The *in vitro* egg hatch test was performed using thiabendazole.

Results: Using Mini-FLOTAC for egg count determination, a 95% of the screened goats were infected with gastrointestinal helminths, and all positive animals were shedding eggs of strongyle nematodes with 92% of strongyle larvae representing *Haemonchus* spp. Strongyles, *Strongyloides papillosus* and *Skrjabinema ovis* were the nematode eggs identified under microscope. Paired and unpaired faecal egg count reduction test calculations detected reductions of 80/81% and 74/73% with samples taken at day 8 and 14 after albendazole treatment, respectively. Albendazole was inconclusive against *Strongyloides papillosus*. *Haemonchus* spp. third stage larvae was the only nematode present after treatment based on coprocultures. The ED_{50} in the egg hatch test was 0.12 µg/ml thiabendazole. There is a reduction in albendazole efficacy in goats in Dagrase, and consequently the development of benzimidazole resistance.

Keywords: Benzimidazole resistance, *Haemonchus contortus*, Goats, South Darfur State, Sudan

UNDER PEER REVIEW

Highlights:

- *Haemonchus contortus* was detected as the predominant gastrointestinal nematode.
- Reduced albendazole efficacy in tested goats.
- The egg hatch tests confirmed the finding of a reduction of albendazole efficacy.
- Only *H. contortus* larvae were present in post-treatment coprocultures.

1. Introduction

Infection with gastrointestinal nematodes (GINs) in sheep and goats is a global threat, particularly in tropical regions, including Sudan. The economic impact due to infection with GINs is high, and was estimated in some countries, such as Ethiopia, as several million dollars annually [1, 2]. In several studies from different regions, *Haemonchus* spp., *Trichortrongylus* spp., *Cooperia* spp., *Nematodirus* spp. and *Oesophagostomum* spp. have been identified as the most frequent nematode genera known to affect sheep and goats, but the most pathogenic effect has been due to *Haemonchus contortus* [3-5]. Infection with *H. contortus*, particularly in lambs and kids, cause anaemia, hypoproteinaemia, reduce weight gain, and death when heavy infection occurred [5].

Control of GINs infection has been performed, and for several decades, by the routine and often frequent use of anthelmintics [6]. Benzimidazole class (e.g. albendazole) has been known as one of the most marketed anthelmintics that used in the treatment and control of GINs for several decades [7]. Due to prolonged use and misuse of anthelmintics, the efficacy of these drugs has been reduced against parasitic helminths of human and animals in parts of the world, and consequently the development of anthelmintic resistance [6]. Nowadays, anthelmintic resistance remains a major threat to the maintenance of livestock in most regions of the world, and has been reported widely among the GINs of ruminants [6, 8]. In Sudan, benzimidazole resistance was recently reported in cattle and goats in South Darfur State, and was found to be highly correlated with the abomasal nematode *H. contortus*. The faecal egg count reduction test (FECRT) revealed an efficacy to albendazole, in resistant areas, from 82 – 94% and 74 – 90% in cattle and goats, respectively [4, 9, 10]. The economic impact due to the development of anthelmintic resistance

UNDER PEER REVIEW

was estimated in 18 European countries as 38 million euros annually [11]. Accordingly, efforts should be required to overcome the spread of this problem, basically, by the regular monitoring of anthelmintic efficacy, then other control strategies adopted, such as the targeted selective treatment (TST) approaches [7, 12].

Goats remain an important resource for poor communities in South Darfur State, where goat population estimated to be around 1.67 million [13]. The present study designed to identify additional benzimidazole resistance population in goats in South Darfur, besides providing some epidemiological data regarding gastrointestinal helminth infections.

2. Materials and methods

2.1. Study area

South Darfur State, southwest Sudan, is a savannah zone where open grazing occurs. The state lies between latitude 11,30ºN and longitude 24,40ºE and has a size of about 127,300 square kilometres. In South Darfur, the rainy season is between June to October with an average rainfall 377 – 546 millimetres annually, with mean minimum and maximum temperature 24.7 and 37.6ºC, and 54.8% mean relative humidity [14]. In this study, Dagrase, a village located 22 kilometres southwest of Nyala, the Capital of South Darfur State, was investigated. This area was studied due to complaints about the poor activity of anthelmintics.

2.2. Study design

The study was conducted in August 2020 in Dagrase with goats (desert goats) naturally infected with GINs. Firstly, male and female goats (*n*=100) of different age groups, from 10 different farms (with 3 – 30 goats/farm, including kids), were screened for the presence of gastrointestinal helminths. Eighty-five goats, of both sexes of different ages (young: <1 year, adult: more than one year based on dentition [15]) that had not been treated in the previous 30 days with an anthelmintic and were shedding >150 strongyle eggs per gram (epg) faeces [16] were selected to study the efficacy of albendazole. Selected goats were randomly grouped into control (*n*=15) and a treated group (*n*=70), that were individually received albendazole (albendazole 10% suspension, Animedica, Livisto, Batch No: E3428202, Germany) at a dose of 10 mg/kg body weight, orally [17]. Animal weights were determined using a 100-kilogram spring balance for

UNDER PEER REVIEW

goats aged up to one year, while for adult's linear body measurement were used [18] as detailed in our previously published article [4]. Faecal egg counts were tested on day 0, 8 and 14 after albendazole administration, respectively. During the study, goats stayed at their farm and remained there after the trial was finished.

2.3. Coproscopic analysis

From the rectum of individual goats, faecal samples were collected in plastic bags, labelled, placed in an isothermal box until counting within 24 h using the Mini-FLOTAC devices with a detection limit of 5 epg [19]. Collected samples were analysed at the laboratory of Parasitology, Faculty of Veterinary Science, University of Nyala, Nyala, Sudan. Pooled faecal samples were collected from control and treated group on day 0 and 14 days after administration of albendazole for microscopic differentiation of strongyle third stage larvae (L3). Samples were incubated for larval cultures for eight days at 22 – 27°C with daily moisturizing with sterile water. The cultures were harvested, using Baermann method, and the first 100 L3 were identified, by genera, morphologically according to Bowman [20].

2.4. Egg hatch test (EHT)

The test was performed as described in the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) [16, 21]. Fresh pooled faecal samples were prepared from strongyle positive goats at farm level on day 0 [21], then nematode eggs were extracted and stored anaerobically until used within three days [22].

2.5. Statistical analysis

The obtained data from the survey for the presence of gastrointestinal helminth infections in goats in Dagrase were used to assess the prevalence of strongyle nematode infection in the tested area using R software version 3.6.1. and the graphical user interface RStudio version 1.3.959. The glm.nb function from the MASS package was used to perform negative binomial regressions for egg count data. As explanatory variables, the sex, the age group (young animals vs. adult) as well as an interaction between sex and age group were considered. Initially, a full model with all potential explanatory variables mentioned above was calculated. Then, the variables were stepwise eliminated using the drop1 function aiming to improve (minimise) the Akaike

UNDER PEER REVIEW

information criterion (AIC). Risk ratios with 95% confidence intervals (CIs) were calculated by applying the confint function on the model coefficients. The RsqGLM function from the modEvA package was used to determine Nagelkerke pseudo R2 values.

The epg of faeces was used to calculate the efficacy of albendazole based on the faecal egg count reduction (FECR), the test being calculated i.e. by comparing the treated group epg with the control group epg on days 8 and 14 (unpaired), or by comparing treated group epg pre- (day 0) and post-treatment (day 8 and 14) (paired) [21, 23]. To calculate the FECR with 95% CIs, paired and unpaired, the R package eggCounts version 2.2 was used with zero-inflation option [24].

The arithmetic means of before and after treatment epg counts of faeces were analysed by one-way ANOVA in GraphPrism version 6.01 at a significant level of $P<0.05$.

The efficacy results of the FECRT and the level of anthelmintic resistance was interpreted as recommended by the WAAVP methods [16, 21]. Anthelmintic resistance was considered when the FECR percentage and the upper 95% confidence interval (UCI) was below 95% and lower 95% confidence interval (LCI) was below 90%. A drug classified as susceptible when the FECR higher than 95% and its lower 95% CI was below 90%. Otherwise, the FECRT was considered to be inconclusive.

The dose of thiabendazole that inhibited 50 of larvae hatching (EC_{50}) in the EHT was determined by a four parameter logistic regression model using GraphPrism version 6.01 software. Benzimidazole resistance was considered when the EC_{50} value was higher than 0.1 μg/ml thiabendazole [16, 21].

3. Results

The prevalence of gastrointestinal helminths in goats in Dagrase was described in Table 1. Ninety-five percentages of the screened goats were positive for the infection with gastrointestinal helminths, and all positive animals were shedding strongyle nematode eggs. Strongyles, *Strongyloides papillosus*, *Skrjabinema ovis* and *Moniezia* spp. were the helminth eggs identified under microscope. Larval cultures examination differentiated strongyle L3 of *Haemonchus* spp., *Trichstrongylus* spp. and *Oesophagostumum* spp./*Chabertia* spp., but the highest percentage was being *H. contortus* (92%) (Table 1). A negative binomial regression model was calculated to

UNDER PEER REVIEW

show the potential effects of risk factors on strongyle egg counts. There were no significant (*P*<0.05) interactions between the variables sex and age (Table 2).

The arithmetic means of epg, compared to control group, of albendazole trial in Dagrase were significantly (*P*<0.05) reduced on day 8 and 14 after treatment. The paired statistics of the treated group, before and after treatment, were also significantly different at *P*<0.05 (Table 3).

The results of the FECRT indicated inefficacy of albendazole against gastrointestinal strongyles, and revealed a reduction of 81% and 73% to day 8 and 14, respectively, with unpaired statistics. The paired FECR showed 80% and 74% to day 8 and 14, respectively. The upper and lower 95% CIs of paired and unpaired analysis methods of day 8 and 14 were below 90%. The EC_{50} in the egg hatch test was 0.12 µg/ml thiabendazole (Table 4). Albendazole was inconclusive against *Strongyloides papillosus*. When the results of larval cultures were obtained from treated goats, only *Haemonchus* spp. L3 were identified microscopically.

4. Discussion

The FECRs as well as the EHT data showed that, benzimidazole resistant *H. contortus* are present in goats in Dagrase. This finding extends the reported presence of benzimidazole resistant *H. contortus* population in cattle and goats in four different study areas in South Darfur State (Kass, Nyala, Rehed Al-birdi and Tulus), Sudan [4, 9, 10], and in many other countries in Africa, such as Ethiopia, Kenya and Uganda [25-27]. The highest response to albendazole in goats in Dagrase was 81% (95% CI: 67 – 88%) indicating resistance as defined by the WAAVP [16, 21]. The EHT result supported the above findings [16]. The obtained EC_{50} value, 0.12 µg/ml thiabendazole, was slightly higher than the value set by WAAVP (0.1 µg/ml) [21]. The FECRT and the EHT results in Dagrase were in agreement with our previous published reports in goats from the South Darfur State. In Nyala, the FECRs were in a range of 80 – 94% to 10 mg/kg body weight dose of albendazole, while ranges of 87 – 91%, 87 – 90% and 87 – 92% were reported in goats in Kass, Rehed Al-birdi and Tulus, respectively. The EC_{50} in the egg hatch test in the four areas were from 0.08 to 0.18 µg/ml thiabendazole [4, 9]. As in Dagrase and other parts of South Darfur State, *H. contortus* is the main nematode species involved in anthelmintic resistance in many countries of the world [28-30]. The much higher reproductive potential of *H. contortus* probably explains why resistance has developed first in this species rather than *Trichostrongylus*

UNDER PEER REVIEW

spp. and *Oesophagostomum* spp., vary between species with more susceptible species taking longer to develop resistance [31]. The main reason that *H. contortus* developed resistance in Dagrase as well as in other regions in South Darfur State is possibly related to the use of the albendazole for a long time, more than three decades [32], in addition to the treatment frequency [4, 32]. Papadopoulos, Himonas and Coles [33] reported development of anthelmintic resistance in nematodes of sheep and goats after two treatments per year on some small islands of Greece which they ascribed to the effect of drought and isolation. In South Darfur State, albendazole has been used for more than three decades with an average of three treatments per year, including in the dry season [4, 34]. With eight months of no rain strongyle larvae are unlikely to survive on the ground thus eliminating pasture refugium. Therefore, treating animals during the dry season will mean that the next generation of worms must come from worms surviving treatment in the animals unless some animals are left untreated. Another main factor significantly contributing to the selection of resistant worm populations is the low quality of marketing anthelmintics in some developing countries. Previous studies from Sudan tested the quality of some veterinary anthelmintics and found that, not all anthelmintics used may be genuine and that mean they actually contain considerably less drug, and this will result in under-dosing [35, 36]. In Sudan, there is another issue might be contributed in the development of anthelmintic resistance, estimation of animal body weight. The use of anthelmintics without proper prior estimation of animal body weight has been resulted in under-dosing which increase the occurrence of anthelmintic resistance development [8]. Noteworthy, when Brazilian farmers have been asked to estimate the body weight of sheep they under-estimated weights [37].

Screening the goats for the presence of gastrointestinal helminth infections in Dagrase can provide some epidemiological information. However, the results of risk factor analysis are not representative, since animals tested only in the rainy season and other parameters such as animal body weight, humidity and local temperature were not documented, but the results might be highlighted the state of gastrointestinal helminth infections in the tested area. The prevalence of gastrointestinal helminths was 95%. This finding is in agreement with our previous studies in goats in South Darfur State, that infection with gastrointestinal helminths are commonly occurring in goats in South Darfur [4, 9]. The egg counts in the screened goats revealed a wide variation in values indicating that all positive goats do not need treating, so TST should be

UNDER PEER REVIEW

possible. Furthermore, measuring eye colour (the FAMACHA test) as an indicator of *H. contortus* burden to individually identify goats requiring treatment instead of whole herd treatment and thus reducing anthelmintic use and slow the development of resistance [38, 39].

5. Conclusions

This study provides extended data on the occurrence of benzimidazole resistance in goats in Sudan. *Haemonchus* spp. were identified as the most predominant nematode genera surviving treatment with albendazole, however *Strongyloides papillosus* eggs were reported for the first time after treatment in South Darfur State. The situation of anthelmintic resistance in South Darfur State, as well as other parts of Sudan, is requiring great efforts to understand the extend of the problem, thereafter the control.

Ethical approval

Approval was obtained from the Research and Ethics Committee at the Faculty of Veterinary Science, University of Nyala, Sudan (Ref. UN/FVS/1/34).

UNDER PEER REVIEW

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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UNDER PEER REVIEW

Table 1

Prevalence, arithmetic mean egg counts (range) and coprocultures (%) of gastrointestinal helminths in the faeces of naturally infected goats at Dagaraz, South Darfur State, Sudan, using Mini-FLOTAC technique.

	All animals	Sex		Age	
		male	**female**	**young**	**adult**
Prevalence of the infection					
No. of the tested goats	100	8	92	17	83
No. (%) of the infected goats	95 (95)	7 (88)	88 (96)	15 (88)	80 (96)
No. (%) of goats shedding strongyle eggs	95 (95)	7 (88)	88 (96)	15 (88)	80 (96)
No. (%) of goats shedding both strongyle & *Strongyloides papillosus* eggs	14 (14)	2 (25)	12 (13)	4 (24)	10 (12)
No. (%) of goats shedding both strongyle & *Skrjabinema ovis*	1 (1)	1 (13)	0 (0)	1 (6)	0 (0)
No. (%) of goats shedding both strongyle & *Moniezia* spp. eggs	3 (3)	0 (0)	3 (3)	0 (0)	3 (4)
Goats shedding >150 strongyle egg/gram faeces	85 (85)	7 (88)	78 (85)	14 (82)	71 (86)
Egg count/gram of positive faeces					
Strongyles	2733 (40 – 14880)	3760 (640 – 14880)	2652 (40 – 12680)	3888 (60 – 14880)	2517 (40 – 12680)
Strongyloides papillosus	311 (40 – 800)	480 (160 – 800)	283 (40 – 560)	520 (320 – 800)	228 (40 – 560)
Coprocultures[a] for strongyles (n=3)[b] (%)					
Haemonchus spp.	92				
Trichostrongylus spp.	4				
Oesophagostomum/*Chabertia* spp.	4				

[a] Samples pooled on a regional level. On the day of sample collection, faecal samples pooled and cultured only from goat shedding strongyle eggs. Third stage larvae harvested, strongyle larvae differentiated microscopically.

[b] Three pools prepared, then the mean calculated.

UNDER PEER REVIEW

Table 2

Final negative binomial regression model to identify variables with influence on faecal egg counts with cattle in five different study areas in South Darfur State, Sudan, during the rainy season.

Term		**Estimate**	**Standard error**	***P* value**	**Rate Ratio**	**95% Confidence interval**
Intercept		7.794	0.129	<0.0001		
Age: Ref.: adult						
	Young	0.347	0.313	0.351	1.416	0.701 – 3.349
Sex: Ref. male						
	Female	-0.008	0.520	0.987	0.973	0.178 – 36.567
Age*Sex: Ref: adult; female						
	Young: male	-0.024	1.315	0.985	1.024	0.024 – 8.714

Nagelkerke R^2 = 0.013

UNDER PEER REVIEW

Table 3

Arithmetic means of egg counts (and 95% confidence interval) with cattle naturally infected with strongyle nematodes before and after oral administration of albendazole at dose of 10 mg/kg body weight to the treated groups at five different study areas in South Darfur State, Sudan.

GI nematode	**No. of animals**	**Mean (95% CI)**		
		Day 0	**Day 8**	**Day 14**
Strongyles	C: *n*=15	3680 (1929 – 5431)	2635 (1505 – 3765)	2517 (1382 – 3653)
	T: *n*=70	2463 (1902 – 3023)	478[a,b] (360 – 596)	633[a,b] (505 – 761)
Strongyloides papillosus	C: *n*=5	224 (95 – 354)	264 (181 – 347)	176 (67 – 285)
	T: *n*=9	267 (152 – 382)	44[a,b] (-18 – 107)	9[b] (-12 – 29)

[a] Significantly different (p<0.05) to control on the same day using a Kruskal-Wallis test with Dunn's post hoc test

[b] Significantly different (p<0.05) to day 0 in the same group using a Friedman test with Dunn's post hoc test

ᴛ Significantly different (p<0.05) to day 8 in the same group using a Friedman test with Dunn's post hoc test

UNDER PEER REVIEW

Table 4

Results of the faecal egg count reduction (FECR) (and 95% confidence intervals) and the 50% effective concentration (EC_{50}) in the egg hatch test for goats naturally infected with gastrointestinal nematodes before and after treatment with albendazole, at dose of 10 mg/kg body weight oral, to the treated group in Dagaraz, South Darfur State, Sudan.

GI nematode	**No. of animals**	**Day 8**		**Day 14**		**EC_{50} (µg/ml thiabendazole)**
		FECR (%) unpaired[a]	**FECR (%) paired[a]**	**FECR (%) unpaired[a]**	**FECR (%) paired[a]**	
Strongyles	C: *n*=15 T: *n*=70	81 (67 – 88)	80 (79 – 81)	73 (58 – 83)	74 (73 – 75)	0.12 (0.04 – 0.34)
Strongyloides papillosus	C: *n*=5 T: *n*=9	76 (14 – 94)	75 (30 – 91)	94 (46 – 100)	96 (83 – 100)	

[a]FECRs were calculated either by comparing data post treatment between treatment and control group (unpaired) or between data before and after treatment (paired).

UNDER PEER REVIEW