Anthelmintic Resistance of Gastrointestinal Strongyles Infecting Sheep in Nziih, West Cameroon

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Authors' contributions

Original Research Article

ABSTRACT

Aims: This study aimed to investigate the levels of efficacy of anthelmintics that were most commonly used in sheep farms in Nziih locality.
Study Design: Longitudinal prospective study.
Place and Duration of Study: The study was carried out in Nziih locality in August 2019 and September 2020.
Methodology: Resistance against Albendazole (12.5 mg/kg) and Ivermectin (0.2 mg/kg) was evaluated using faecal egg count reduction test in naturally occurring gastrointestinal strongyles of sheep in Nziih. A total of 300 young animals were randomly selected from 5 farms in 2019 and in 2020. Faecal samples were collected from each animal on the first day of treatment (D0) and collected again 14 days later (D14). Faecal Egg Counts were performed using the Willis and Mac Master techniques and resistance was declared when faecal egg count reduction (FECR) was less than 95 % and the lower limit of confidence interval (LCI) less than 90%. Pooled faecal samples for each treatment group were set for D0 and D14 to identify resistant strongyles genera by calculating persistent larval rate (PLR).
Results: Resistance against Ivermectin was detected while there was none against Albendazole. Moreover, it was declared only in 2 farms out of the 5 selected. From 2019 to 2020, the status of

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resistance has shifted from suspicion to resistance for ivermectin and from absence of resistance to suspicion to Albendazole. Resistance was recorded in 2 sites: Pastorale1 (FECR= 85 %, LCI= 42.61 %) and Pastorale 3 (FECR=90.5 %, LCI= 72.26 %) in large flock herds and in farms where sheep were dewormed at least 4 times per year. *Trichostrongylus* were more persistent than *Haemonchus* and *Oesophagostomum/Chabertia* with PLR of 21.22 %, 13.71 %, and 7.40 % respectively.
Conclusion: Anthelmintics must be used correctly in conjunction with farmer education in Nziih.

1. INTRODUCTION

Gastrointestinal nematode parasites are one of the major threats to animal husbandry especially, strongyles which are very common and may cause severe infection [1]. They are responsible for considerable morbidity and mortality in small ruminants due to impairment in body weight gain, reduction in milk and wool production, anaemia, anorexia, malabsorption, and diarrhoea which result in a loss in quantity and quality of animal carcass [2,3] This leads to a decrease in productivity and profitability of this income-generating activity especially for smallholder farmers [4]

Anthelmintics have been used to fight against these infections. However, long-term, repeated and misuse of a limited range of these drugs precedes farmers' complaints about the persistence of symptoms after treatment. Worms have indeed developed some resistance to anthelmintics [5,6]. In Africa, the control of these parasites faces problems of availability and cost of drugs. The use of cheap generic drugs of dubious quality is very common and contributes to the development of resistance to the parasites reported on the continent [7,8]. Defined as the inherited ability of a helminth to survive a normally effective dose of anthelmintic, resistance concerns all families of anthelmintics and are now a concern for all livestock farms worldwide [9]. There is a rising issue encountered in tropical countries but recent studies have demonstrated that there is an alarming situation in most European countries too which are increasingly being exposed [10,11]. Indeed, over the last three decades, anthelmintic resistant worm populations have been reported in most countries where sheep farming is a significant part of the agricultural economy [9]. In Africa, the overall prevalence of anthelmintic resistance (AR) has not been extensively investigated, particularly within the resource-poor farming sector, but resistance has been reported from at least 14 countries with most of the reports emanating from Kenya and South Africa [12]. Benzimidazoles resistance has been reported in Cameroon precisely in South West and Adamawa regions [13,14]. However, there is no data on anthelmintic resistance of ovine gastrointestinal strongyles in the Western part of the country where small ruminant husbandry represents a significant part of the economy of the rural population. As reported by Makamte *et al.* [15], ovine gastrointestinal nematodes infection represents a threat in Nziih and their control relies exclusively on the use of anthelmintics. In such farming systems, emergence of AR often poses a severe threat to livestock production. Besides, genes conferring AR are thought to be present in a small proportion of individuals in the population even before the worms are exposed to a drug for the first time [12]. Following the complaints from breeders about the inefficacy of anthelmintics, it was timely to verify this assertion.

The objective of the present study was to investigate the levels of efficacy of anthelmintics that were most commonly used in sheep farms in Nziih locality (West region of Cameroon). the development of AR being inevitable and its occurrence not being a matter of "if" but "when" as declared by Bosco *et al.* [16].

2. MATERIALS AND METHODS

This study was conducted at Nziih in "Bafou", Nkong-ni subdivision, Menoua Division in the West Region of Cameroon. Nziih villages are situated between 8'10° latitude North and 5'8° longitude East. Altitude is between 800 m and 2740 m on red ferralitic and volcanic soils. Vegetation is made up of shrub savanna with galleries modified by anthropic action. Climate is of the soudano-guinean type and consists of two seasons: the rainy season (March to November) and the dry season (December to February). Precipitation varies between 1910 to 2000 mm from March to November and temperature varies from 14.3°C to 23.4°C annually. Humidity varies

from 40% in the dry season to 100% during the rainy season. Pastures are abundant in the rainy season and several forage species are found. The hydrographic network is made up of a few rivers and springs that may dry up. The economy of the area is based on Agriculture. [17,18,19] The map of the data collection site is shown in Image 1.

2.2 Sample and Data Collection

2.2.1 Farms selection

The study was carried out in August 2019 and September 2020. Five sheep farms (Mezet 2, Feumock, Pastorale 1, 2 and 3) were selected based on their anthelmintic resistance (AR) risk as indicated by farm history and knowledge of repeated treatment each year (>2). On each farm, the breeder was questioned regarding the type, frequency of treatment, dose, method of administration, and place of origin of anthelmintic.

2.2.2 Experimental design

The procedure to detect anthelmintic resistance in strongyles was that recommended by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) [20]. Animals of 3 to 6 months were randomly selected from those that had at least 150 eggs per gram of faeces (EPG) and had not received any anthelmintic treatment at least 2 months prior to the start of the study. On each of the five farms, 30 animals (ten per group) were sampled and identified by a collar bearing the animal's identification number. The following treatments were assigned day 0 using the dose rates recommended by the manufacturers: (1) untreated group, (2) Albendazole (Albenvet®), given orally at 12.5 mg/kg Body Weight by a syringe of 20 ml and (3) ivermectin (Cevamec®) administered subcutaneously at 0.2 mg/kg Body Weight. Each group was made of 10 animals. Drugs were administered to all animals in the group with the dosage based on their weight. Faecal samples were taken from each animal on D0 and D14 post-treatment. The diagnostic technique used to monitor the faecal egg excretion was Mac Master technique [21]. The Faecal Egg Count Reduction was determined using the formula of Coles *et al.* [20]

$$FECR = (1 - \frac{FECt}{FECc}) \times 100$$

Where FECt is the arithmetic mean of individual faecal egg counts in a treated group and FECc is the arithmetic mean of individual faecal egg counts in the control group. According to the WAAVP guidelines, resistance is present if the percentage reduction in egg count is less than 95% and the lower limit of the 95% confidence interval is less than 90%. If only one of the two criteria was met, then the finding was recorded as suspected anthelmintic resistance.

2.2.3 Cultivation of gastrointestinal nematode infective larvae

Pooled faecal samples from each treatment group (1 g per animal) were mixed with sterile sheep faeces until the mixture was not too grievous or too liquid. Then, each jar containing the mixture was placed in an incubator for 10 days at 25 °C and humidified with tap water when necessary. This was set for each farm, in each group and at D0 and D14, representing 60 larval cultures (5 farms x 3 groups x 2 dates x 2 times) both in 2019 and 2020. Third-stage larvae (L3s) were recovered by removing each faecal culture from its jar and placed on a tea strainer covered with a filter paper. The set was then placed on a jar filled with tap water to its 4/5th and incubated at 25 °C for 24 hours to allow the infective larvae to migrate from faecal suspension to the water. After this, the tea strainer and the three-quarters of larval suspension present in the cup were removed. The remaining one quarter was transferred to a graduated test tube and left to stand for 10 minutes. Then, the supernatant was poured off and only one ml was kept at + 4 ° C for identification of L_3 [22]. Morphological identification of L_3 was based on the total length of larvae, the tail-length larvae, and the presence or the absence of two cranial refringent bodies. It was set by observing under the microscope one drop of larval suspension appended to one drop of Lugol solution [23]. The concentration of larvae per milliliter in a larval suspension was evaluated by counting all gastrointestinal strongyle larvae under a binocular microscope at 100 X magnification within 0.5 ml [15]. Persistent larval rate (PLR) was calculated after treatment to know which genera are resistant to drugs following the formula:

$$PLR = \frac{Number\ of\ larvae\ after\ treatment}{number\ of\ larvae\ before\ treatment} \times 100$$
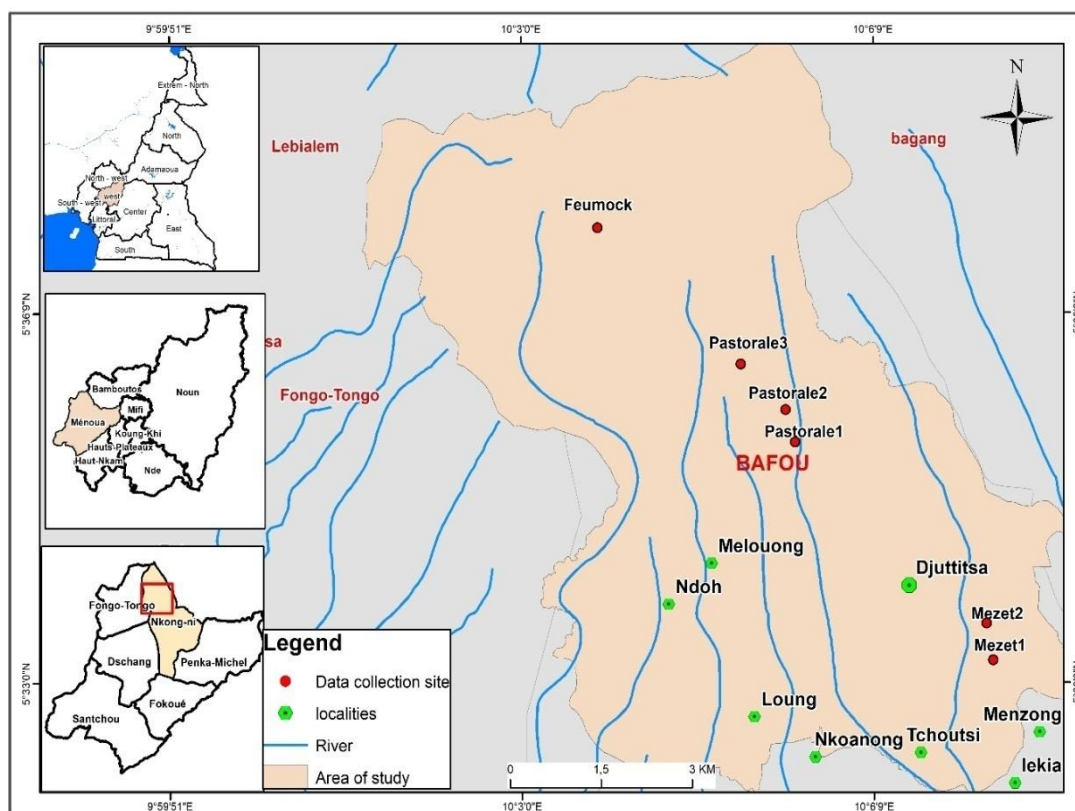
Image 1. Map of Bafou North area (Nziih)

2.3 Statistical Analysis

The data obtained from the study were recorded in Microsoft excel 2016 software and transferred for analysis to Statistical Package of Social Sciences (SPSS) software version 22.0. Persistence larval rates of strongyle genera for the 2 years were compared using the Kruskal Wallis. PLR after treatment with Albendazole and Ivermectin were compared using Student-t-test. The confidence level was held at 95 %.

3. RESULTS

3.1 Anthelmintic Treatment Profile in Nziih

The questionnaires in the different farms revealed that anthelminthic treatments were given on average 2 to 3 times a year with Ivermectin being predominant, followed by Albendazole and Levamisole. Also, drugs were bought in the livestock market rarely in a veterinarian pharmacy, and treatments were administered by farmers. Anthelmintic treatment profile at Nziih is described in Table 1.

3.2 Anthelmintic Resistance Profile

A total of 300 animals were included in the study. The anthelmintic efficacy test revealed that no resistance or suspected resistance to Albendazole was detected in any of the farms in the year 2019. However, in 2020, the only suspected resistance to Albendazole was revealed at Pastorale (the lower limit of the confidence interval of the FECR was less than 90 %). In 2019, gastrointestinal strongyles were suspected to be resistant to ivermectin in Feumock, Pastorale 1, and Pastorale 3. While in 2020, ivermectin resistance was detected in Pastorale 1 and Pastorale 3 with the FECR of 85 % and 90.5 % respectively. Faecal egg count reduction (FECR) and their confidence intervals per farm are presented on Tables 2 and 3.

3.3 Larval Counts and Identification

The pooled coprocultures pre-treatment and post-treatment provide further insights into which strongyles genera are potentially resistant or not to the anthelmintics tested. Thus, the persistent larval rates (PLR) were calculated in 2019 and 2020. The albendazole PLR was under 5 %, but

it varied from 0 to 21.22 % after treatment with the ivermectin. The ivermectin post-treatment PLR was greater than of Albendazole (P=0.008). However, the generic PLRs were statistically different (P=0.04). The larvae belonged to *Trichostrongylus*, *Haemonchus* and *Oesophagostomum/Chabertia* genera. The PLR in 2019 and 2020, after treatment with Albendazole and Ivermectin are presented in Figs. 1 and 2 respectively.

Table 1. Anthelmintic treatment profile in Nziih

Farms localization	Number of animals		Treatment (frequency along a year)	
	2019	**2020**	**2019**	**2020**
Mezet 2	72	64	IVM(2/year), ALB (2/ year)	IVM (2/ year) ALB (2/ year)
Feumock	60	70	IVM (3/ year), ALB (1/ year)	IVM (3/ year) LEV (1/ year)
Pastorale 1	143	175	IVM (3/ year), ALB (1/ year)	IVM (3/ year) ALB (1/ year)
Pastorale 2	102	99	IVM (1/ year), ALB (1/ year)	IVM (2/ year) ALB (0/ year)
Pastorale 3	200	212	IVM (4/ year), LEV (1/ year)	IVM (4/ year) ALB (1/ year)

ALB: Albendazole; IVM: Ivermectin; LEV: Levamisole

Table 2. Faecal egg count reduction rate and confidence interval at 95 % in 2019 at Nziih

Group	Results	Mezet 2		Feumock		Pastorale 1		Pastorale 2		Pastorale 3	
		J0	**J14**	**J0**	**J14**	**J0**	**J14**	**J0**	**J14**	**J0**	**J14**
Control	FEC mean (EPG)	560	770	1210	1150	1630	1320	1220	1180	3000	2660
ALB	FEC mean (EPG)	400	0	720	10	1740	10	380	0	2960	5
	FECR (%)	100		99.13		99.24		100		98.12	
	CI	-		92.83-99.14		93.67-99.91		-		98.17-99.98	
IVM	FEC mean (EPG)	450	0	470	20	1170	40	1600	0	1620	55
	FECR (%)	100		98.26		96.96		100		97.93	
	CI	-		85.98-99.79		74.69-99.64		-		83.31-99.74	

ALB: Albendazole; IVM: Ivermectin; FEC: Faecal Egg Count; FECR: Faecal Egg Count Reduction; CI: Confidence Interval

Table 3. Faecal egg count reduction rate and confidence interval at 95 % in 2020 at Nziih

Group	Results	Mezet 2		Feumock		Pastorale 1		Pastorale 2		Pastorale 3	
		J0	J14	J0	J14	J0	J14	J0	J14	J0	J14
Control	FEC mean (EPG)	1730	1910	660	800	690	800	370	460	1270	1110
ALB	FEC mean (EPG)	4420	0	590	0	1240	10	1420	0	570	0
	FECR (%)	100		100		98.75		100		100	
	CI	-		-		89.69-99.84		-		-	
IVM	FEC mean (EPG)	680	50	590	70	1340	120	600	15	1740	105
	FECR (%)	97.38		98.26		85		96.73		90.5	
	CI	85.12-99.54		68.60-97.54		42.61-96.04		85.42-99.27		72.26-96.74	

ALB: Albendazole; IVM: Ivermectin; FEC: Faecal Egg Count; FECR: Faecal Egg Count Reduction; CI: Confidence Interval
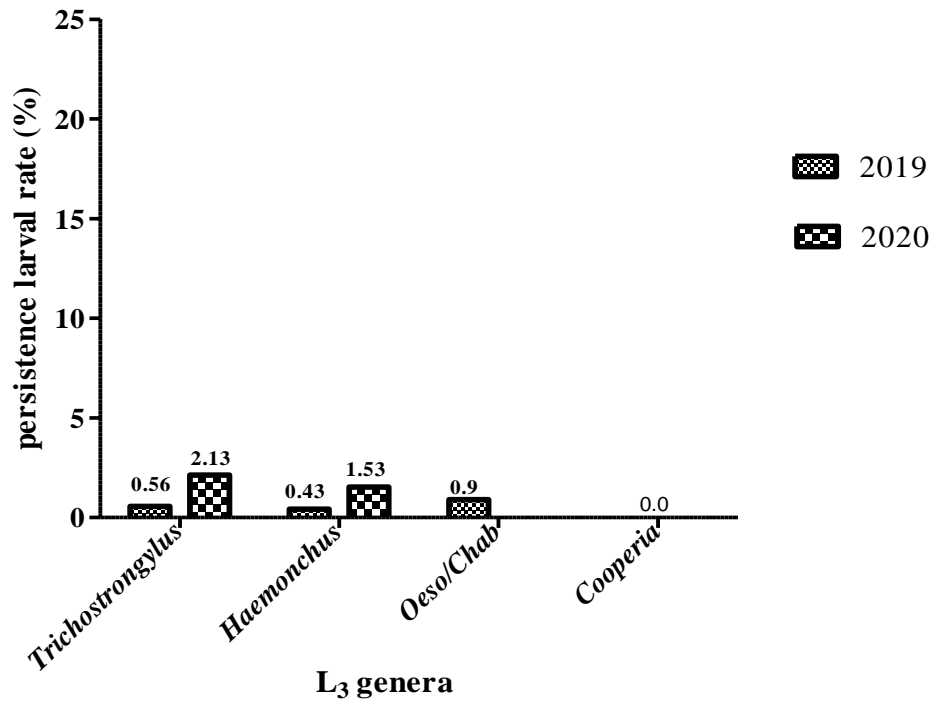
Fig. 1. **Persistent larval rate after treatment with Albendazole in 2019 and 2020**
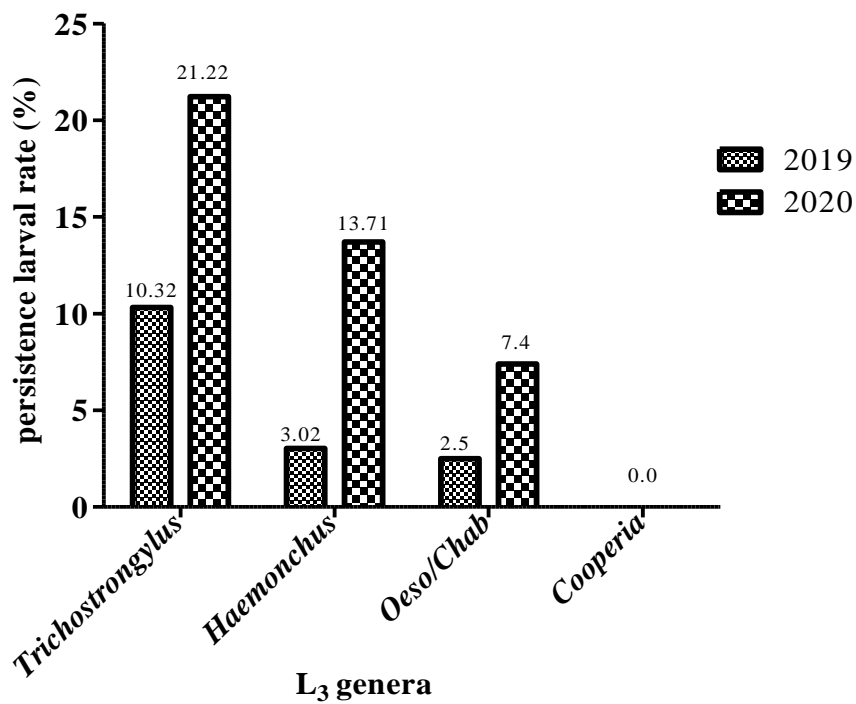*Oeso/Chab: Oesophagostomum/Chabertia*

Fig. 2. Persistent larval rate after treatment with Ivermectin in 2019 and 2020
Oeso/Chab: Oesophagostomum/Chabertia

4. DISCUSSION

Ivermectin, Albendazole, and Levamisole were the most commonly used anthelmintic drugs in the study area. this was also the case in South Africa [24]. Ivermectin was the most commonly used anthelmintics because it is cheap. Also known as "endectocide", ivermectin is used by farmers for its action against both ectoparasites (mites and ticks) and endoparasites. Albendazole was the second most used drug certainly due to its low cost. In fact, drugs of benzimidazole class are generally cheaper than other classes of anthelmintics [24,14].

The faecal egg excretion reduction test was used to assess the effect of anthelmintics on the strongylids population in Nziih by identifying cases of suspicion and resistance. Only the strongyles from Pastorale 1 and Pastorale 3 revealed resistance to ivermectin with the highest persistent larval rate (PLR) for *Trichostrongylus* and *Haemonchus*. These results are in agreement with Bosco *et al.* [16] in Italy. However, they differ from many studies carried out in Africa such as those of Sassa *et al.* [14] in Cameroon, Mohamedsalih *et al.* [25] in Sudan and Mphahlele *et al.* [26] in South Africa which reported the resistance of *Haemonchus* species benzimidazole in small ruminants. A low level of anthelminthic drug alternate was observed: one treatment with Albendazole or levamisole against 2, 3 or even 4 treatments with ivermectin. Indeed, frequent use of an anthelmintic exerts selection pressure on the parasites and therefore constitutes a major risk of the emergence of resistant worm populations [27]. On the contrary, the use of two different anthelmintic classes concurrently may delay the development of anthelmintic resistance and when animals are treated with two different classes simultaneously resistance doesn't develop for over 20 years [28]. The higher the frequency, the stronger the selection and the more resistant worms become. Similarly, the use of a single anthelmintic molecule even at a low frequency is a source of resistance development [29,9].

Moreover, large herd size has been reported as a risk factor for the presence of resistance, this is may also be the reason why resistance was observed in Pastorale 1 and 3 which had the highest herd size. Indeed, farmers with large flocks are more likely able to buy anthelmintics. Conversely, farmers with smaller flocks often cannot afford to buy drugs and this might slow down the onset of resistance [12].

The resistance phenomenon observed may also be due to the fact that anthelmintic drugs were of dubious origin because not only were they purchased at the livestock market but also the labels on the bottles did not show the name of any veterinary laboratory. Such drugs are therefore reportedly less effective than those from licensed outlets. In addition, they are administered by the farmers themselves who did not have control over the doses prescribed by the manufacturer. They had recourse to veterinarians for intervention only when there were many cases of mortality. In reality, dosing errors, especially under-dosing, favor the selection of chemoresistant parasites. In fact, only susceptible individuals (SS homozygotes) in the parasite population are affected; resistant individuals (SR heterozygotes and RR homozygotes) survive and are the source of subsequent resistant generations [30,31]. The emergence of resistance in Nziih could be due to the introduction of purchased sheep harboring resistant worms from other Regions (South West and North) of the country into the flock. Several authors have shown that the occurrence of resistance is due to the introduction of resistant worms from other herds, either through the sale of animals or through the existence of common pastures [12].

The resistance mechanism of macrocyclic lactones, to which ivermectin belongs, is due to an overexpression of genes coding for P-gp efflux pumps [32]. Furthermore, the low prevalence of resistance (2/5) in this area is probably due to the low income of farmers who regularly carry out targeted treatments according to the symptoms presented by the animal like diarrhoea, inappetence, weakness and weight loss.

The efficiency of Albendazole in the study area is due to the low frequency of use of this molecule compared to ivermectin. The selection pressure of Albendazole is still very low since there is always a very small proportion of worms in a helminth population with a genetic aptitude to resist anthelmintics [12, 27]. Moreover, the proportion of parasites carrying the resistant allele would be very low since the loss of efficacy of an anthelmintic becomes clinically apparent when 20% of individuals are homozygous resistant [9].

5. CONCLUSION

Ovine strongyles in Nziih, have developed resistance to ivermectin which is the most

commonly used anthelmintic in the area. The anthelmintic resistance to the genera *Trichostrongylus, Haemonchus*, and *Oesophagostomum/Chabertia* was observed in the farms. However, Albendazole is effective. These results show a need for implementing urgent measures to slow down the development of resistance to reduce the frequency of use of ivermectin and to respect the doses of anthelmintic drugs.

DISCLAIMER

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.

CONSENT

After exposing the objectives of the study, consent was obtained from all owners prior to faecal sample collection which was done without any harm.

ETHICAL APPROVAL

This work was carried out in accordance with the Animal Ethical Committee of the Animal Biology Department of the University of Dschang, Cameroon. The authorization to carry out the study was given by the Departmental delegate of the Ministry of Livestock, Fisheries and Animals Industries (MINEPIA) of the Menoua Division.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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