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Assessing anthelmintic resistance on small ruminant farms in a tropical production system

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ABSTRACT

Imidazothiazoles and benzimidazole are the only classes of anthelmintic drugs that have been used over the past 40 years in Fiji. Recently, concerns have arisen that anthelmintic resistance could be widespread and affect animal health and productivity in Fiji. The present study was designed to evaluate the current anthelmintic resistance status in Fiji's small ruminant farms. The study included 11 farms from the two (Western and Northern divisions) most relevant areas of small ruminant production in Fiji. The anthelmintic treatments tested were levamisole (LEV), albendazole (ALB), levamisole + albendazole combination (LEV+ALB), ivermectin (IVM), moxidectin (MOX), closantel (CLO) and a negative control (CON). The anthelmintic's efficacy was tested using faecal egg count reduction (FECR) tests and copro-cultures on days 14, 28, and 42 after treatment administration. The lowest mean FECR on day 14 was observed for ALB (65.2 %) followed by LEV (91.6 %), ALB + LEV (94.3 %), IVM (97.4 %) and MOX (98.8 %). The most relevant genera of GIN encountered were *Haemonchus* and *Trichostrongylus* spp., with no distinct pattern of resistance to drug groups between the two populations. None of the tested drugs (MOX and CLO) presented FECR over 95 % on days 28 and 42. Overall, the level of anthelmintic resistance observed was lower than hypothesised for this study. The combination of LEV+ALB has proven effective and presents an appealing treatment option for managing anthelmintic resistance and worm burden reduction in Fiji.

1. Introduction

Gastrointestinal nematodes (GIN) significantly reduce the productivity of grazing animals, especially in tropical and subtropical areas. In these environmental conditions, small ruminants (SR) are highly susceptible to GIN infections because high temperatures and rainfall favour the hatching and development of free-living stages of GIN (Waller, 1997), shortening the life cycle, and contamination levels can increase rapidly (Barger et al., 1994). These larvae have a short survival time in the tropics (as little as 5–13 weeks) due to environmental factors (e.g. temperature and moisture) compared to up to 12 months in temperate regions (Banks et al., 1990; Barger et al., 1994; O'Connor et al., 2006).

The main method for controlling GIN is by anthelmintic administration, given the practicality and relatively low cost (Fissiha and Kinde, 2021; Potárniche et al., 2021). However, anthelmintic resistance is a growing concern for SR farmers worldwide. Resistance occurs when the efficacy of anthelmintic treatment declines due to evolutionary

principles of selection and fitness. Factors such as frequency and level of exposure to anthelmintic classes, underdosing and long-acting preparations can influence the development of resistance since it exposes the GIN population for a longer time to the actives (Abbott et al., 2012; Leathwick and Besier, 2014).

Using a single anthelmintic against multiple resistant strains would rapidly increase the resistance level. On the other hand, a combination of anthelmintics can be more effective against worms that are resistant to each component when administered individually (Le Jambre et al., 2010). Resistance has been detected for all anthelmintic classes used for SR (Kaplan, 2004). Detectable resistance to new anthelmintic classes is typically observed in parasites within 5–8 years of the introduction of a class (Kaplan, 2004; Fissiha and Kinde, 2021).

The GIN species *Haemonchus contortus* and *Trichostrongylus colubriformis* are prevalent in tropical and subtropical regions and exhibit resistance to a wide range of anthelmintics (Walkden-Brown and Banks, 1986; Banks et al., 1990; O'Connor et al., 2006; Arsenopoulos et al.,

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2021). In Fiji, the most common GIN species previously reported in sheep and goats include *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., and *Strongyloides* spp. (Walkden-Brown and Banks, 1986). Over the past 4 decades, only two classes of anthelmintics have been available for use on Fijian SR farms. The misuse of these 2 classes over the years may have led to resistance against benzimidazoles (primarily albendazole, ALB) and imidazothiazoles (specifically levamisole, LEV). Additionally, a third class, macrocyclic lactones (ivermectin, IVM), is used exclusively at government research stations. The precise initiation year for the use of anthelmintics in Fiji remains uncertain. Nonetheless, the earliest documented investigation into GIN control in Fiji pertains to the work of Baker (1970), who emphasised rotational grazing as a strategy to prevent the development of AR.

A faecal egg count reduction (FECR) test conducted on 24 farms in 1986 revealed that goats and sheep on Fiji's farms already exhibited some resistance (i.e. FECR < 95 %) to ALB (8/18 farms, 44 %) or LEV (8/21 farms, 38 %) but not IVM (0/9 farms, 0 %) (Banks et al., 1987). Fifty-four per cent (13/24) of the surveyed farms showed resistance to either fenbendazole or LEV, while 17 % (3/18 farms) were found to have resistance to both fenbendazole and LEV (Banks et al., 1987). Despite being suspected, as some farmers purchased animals from the government research stations where IVM was used, no resistance to IVM was detected (Banks et al., 1987). Cowley et al. (2019) conducted a survey across several farms in Fiji and measured worm burden levels of > 1400 epg and > 2400 eggs per gram (epg) in sheep and goat farms, respectively. In addition, farmers reported drenching their animals at regular intervals, an average of every 1.6 months (~48 days). Given the historic utilisation of these drugs, these results led to the hypothesis that anthelmintic resistance would be detected for the current (LEV) and previously commercialised (ALB) drugs. In contrast, unused drugs such as IVM, MOX, and CLO, as well as the combination of ALB+LEV, would provide high efficacy in reducing faecal egg counts (FEC). Ivermectin, MOX, and CLO were never commercially available to SR farmers in the country but were used at government research stations. There are two primary reasons for testing unused drugs. First, these drugs could be valuable in the future if resistance to current medications emerges. Second, they are commonly utilised at government research stations and serve as a source for farmers who purchase animals from these facilities. A combination of LEV+ALB was also tested (Le Jambre et al., 2010; Wormboss, 2024) to explore future options with existing anthelmintics.

2. Material and methods

2.1. Ethical approval

The participating farmers were informed about the study, and consent was obtained before the trial started. The University of New England's animal ethics committee authorised the study (ARA 21/110).

2.2. Study location and climate

Fiji is a tropical island country located between latitudes 15 °S and 22 °S and longitudes 174 °E and 177 °W; it covers 18,378 km² of land scattered over 230,000 km² of the ocean (Twyford and Wright, 1965). Together, the two main islands of Viti Levu and Vanua Levu make up 87 % of the country's total land area (Twyford and Wright, 1965). Throughout the country, the annual rainfall varies from 1800 to 3500 mm (Kumar et al., 2014). According to the Köppen climate classification, Fiji has a tropical rainforest and monsoon climate (Arnfield, 2020). It is usually described as having only two distinct seasons during the year: dry and wet. The dry season usually lasts from May to October and the wet season from November to April. The study was conducted in Fiji's Western and Northern Divisions from February to April 2022 and May to July 2022, respectively.

2.3. Selection of farms, animals and anthelmintic treatments

Among the Pacific Island countries, Fiji has the largest SR population, estimated at 37,435 sheep and 143,853 goats in 2020. According to the Fiji National Agricultural Census (Ministry of Agriculture, 2020) 4341 households are involved in sheep farming and 9212 in goat farming, with most of the country's SR population (sheep 97.2 % and goats 93.2 %) located in the Western and Northern administrative divisions. The 11 farms selected to participate in this trial were all privately owned from the Western (n = 5) and Northern (n = 6) divisions of Fiji (Table 1). The selected farms had not administered anthelmintic treatment for at least 60 days before the beginning of the trial, however, most farms (7 out of 11) in this study have reported a history of drenching frequency of more than 10 times a year.

The anthelmintic treatments tested were LEV [levamisole hydrochloride 27 g/L; a dose of 12 mg/kg liveweight (LW, goats) and 6.5 mg/LW (sheep)] and ALB[ALB; albendazole 19 g/L; dose of 3.8 mg/LW (sheep and goats)], two anthelmintics used in Fiji for many decades; a combination of ALB and LEV [same products as single action drugs; dose of 3.8 + 12.0 mg/kg LW (goats) and 3.8 + 6.5 mg/LW (sheep)], as well as anthelmintics not previously used on Fijian farms: IVM [ivermectin 0.8 g/L; dose of 0.4 mg/kg LW (goats) and 0.2 mg/kg (sheep)], moxidectin [MOX; moxidectin 1 g/L; dose of 0.4 mg/kg LW (goats) and 0.2 mg/kg LW (sheep)], closantel [CLO, closantel 37.5 g/L, 10 mg/kg (goats) 7.5 mg/kg (sheep)] and a negative control (CON) group in all farms. All 7 anthelmintic treatments could not be applied on all farms due to insufficient flock sizes, so each anthelmintic treatment was applied on 7 of the 11 farms in different groupings: All treatments were tested together on 3 large farms (2 in the Western and 1 in the Northern Division), whereas LEV, ALB, and LEV+ALB were tested together on one set of small farms and IVM, MOX, and CLO were tested on a different set of small farms (Table 1). The experimental design and treatments are summarised in Table 2. The target group size was 10 animals (sheep and goats), consistent with the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for the detection of anthelmintic resistance in nematodes (Coles et al., 2006; Burden et al., 2024), but this was not achieved for all groups, with a minimum group size of 6 occurring in one case (Table 2). The selected animals were growing un-castrated male and female animals over 15 kg and were randomly selected for inclusion, with an average live weight (mean ± SD) of 25.2 kg ± 6.0 and 31.9 kg ± 8.8 for goats and sheep, respectively. The doses were selected based on the label claim of each product or suggested in the literature (Terrill et al., 2001; Dixit et al., 2019). All anthelmintics were administered orally using a syringe on day 0, with dosages individually calculated for each animal based on live weight (Table 2).

2.4. Faecal egg count and larval differentiation

Individual faecal samples were obtained per rectum on day 0 (pre-treatment), post-treatment day 14 (all treatments), and post-treatment days 28 and 42 for the CLO, MOX and their respective control groups. The faecal samples were analysed for FEC by a modified McMaster technique (Kennedy, 1982) using ~2 g of faeces mixed with 28 ML of saturated salt solution, with a detection limit of 50 epg. Copro-culture was carried out on pooled samples from each treatment group on each farm on days 0 (pre-treatment), 14, 28, and 42 (post-treatment). Vermiculite was used in a 1:1 ratio, with approximately 50 g of faeces comprising a pool derived from ~5 g from each animal in the treatment. The cultured bottles were incubated in air-forced laboratory incubators (MRC DFI 80, United Kingdom) aerobically at 27 °C for seven days (Fisheries, 1986). The recovery of larvae was performed using the inversion method (Berrie et al., 1988). Identification of the third stage larvae (L3) was then performed under a microscope at a magnification of 40 × following staining with Lugol's iodine using the standard keys to the genus level of each larvae (Kennedy, 1982; Van Wyk et al., 2004).

Table 1
Summary description of the small ruminant farms included in the study.

Farm	Location	Type of farm ^B	Host species	Herd/Flock size	Farming experience (years)	Initial FEC (EPG)	Treatment group ^A
1	Northern	Goats	Goats	60	12	374	1
2	Northern	Sheep and Goats	Sheep	57	9	1925	1
3	Western	Goats	Goats	59	15	1470	1
4	Western	Sheep and Goats	Sheep	57	3	685	1
5	Northern	Goats	Goats	90	10	270	2
6	Northern	Sheep	Sheep	57	13	1784	2
7	Western	Sheep	Sheep	55	6	1938	2
8	Western	Sheep and Goats	Goats	278	20	2261	2
9	Northern	Sheep	Sheep	93	17	1476	3
10	Northern	Sheep and Goats	Goats	80	9	429	3
11	Western	Goats	Goats	143	7	2368	3

Small ruminant (SR); Faecal egg count (FEC); Egg per gram (EPG).

^AGroup 1: Levamisole, Albendazole, Levamisole + Albendazole and Control;

^AGroup 2: Ivermectin, Moxidectin, Closantel and Control;

^AGroup 3: Levamisole, Albendazole, Levamisole + Albendazole, Ivermectin, Moxidectin, Closantel and Control;

^BMixed farms rear sheep and goats in separate paddocks and sheds.

Table 2
Summary description of treatment distribution by species and location, treatment group size per farm, average live weight and treatments utilised in the trial on the small ruminant farms in Fiji's Western and Northern divisions.

	Anthelmintic treatments						
	ALB ^A	LEV	ALB+LEV	IVM	MOX	CLO	CON
Total number of farms (n)	7	7	7	7	7	7	11
Total number of animals (n)	55	57	56	63	63	60	89
N of farms tested by division (n)							
Northern	4	4	4	4	4	4	6
Western	3	3	3	3	3	3	5
N of farms tested by species (n)							
Goat	4	4	4	4	4	4	6
Sheep	3	3	3	3	3	3	5
Group size per farm (n)							
Maximum	9	10	10	10	10	10	10
Minimum	7	7	7	7	7	7	6
Average liveweight (kg ± sd)							
Goat	25.3 ± 7.0	23.0 ± 4.6	24.9 ± 6.5	24.6 ± 5.6	27.5 ± 6.1	25.1 ± 6.1	25.7 ± 5.8
Sheep	28.9 ± 7.7	31.3 ± 9.6	33.2 ± 8.2	32.5 ± 9.3	34.0 ± 8.8	31.6 ± 9.8	31.7 ± 8.4
Dosage (mg/kg)							
Goat	3.8 ^B	12 ^C	3.8 ^B + 12.0 ^C	0.4 ^C	0.4 ^C	10.0 ^D	3.8 ^B
Sheep	3.8 ^B	6.5 ^B	3.8 ^B + 6.5 ^B	0.2 ^B	0.2 ^B	7.5 ^B	3.8 ^B
Sampling (days)	14	14	14	14	14, 28, 42	14, 28, 42	14, 28, 42
Products used	Albendazole (Alben, albendazole 19 g/L, Virbac, Australia)	Levamisole (Nilverm Oral®, levamisole hydrochloride 27 g/L, Coopers, Australia)	Albendazole (Alben, albendazole 19 g/L, Virbac, Australia) + Levamisole (Nilverm Oral®, levamisole hydrochloride 27 g/L, Coopers, Australia)	Ivermectin (Ausmectin® sheep drench, ivermectin 0.8 g/L, Australia)	Moxidectin (Cydectin Oral®, moxidectin 1 g/L, Virbac, Australia)	Closantel (WSD Closantel Oral, closantel 37.5 g/L, WSD Agribusiness Pty. Ltd, Australia)	

^A Albendazole (ALB); Levamisole (LEV); Ivermectin (IVM); Moxidectin (MOX); Closantel (CLO); Control (CON)

^B Registered dose and label claim, ^C Reference (Terrill et al., 2001), ^D Reference (Dixit et al., 2019).

A target of 100 larvae to be counted was set, irrespective of the genus, but this was frequently not achieved post-treatment.

2.5. Statistical analysis

All statistical analyses were conducted using JMP® version 16.2.0 software. The pre-treatment (day 0) FEC was calculated numerically

using a mixed least squares model. The interaction and main effects of host species and location were fixed factors, and farm ID was a random factor. Faecal egg count reduction was calculated using the Microsoft® Excel RESO FECR spreadsheet and calculator (Waller et al., 1989) modified to include methods to develop confidence limits (CL) developed by Brown et al. (2001) and Dobson et al. (2012). FECR was calculated for overall FEC and individual genera with upper and lower

95 % confidence limits (Burden et al., 2024). The RESO FECR was calculated by using the following equation:

$$FECR(\%) = 100 \times \left[1 - \left(\frac{T}{C} \right) \right]$$

where T and C are the arithmetic mean FEC of the treated and control group on the same day of sampling. Negative FECR were truncated to a value of zero. The efficacy status of anthelmintics was interpreted based on the WAAVP guidelines (Coles et al., 1992; Kaplan et al., 2023), where resistance is present if (i) FECR is less than 95 % and (ii) the lower 95 % CL is less than 90 %. When only one of these criteria is met, resistance is suspected. The parasite is classified as susceptible if none of the criteria is met. Summary statistics and figures were created using JMP® version 16.2.0 software (SAS Institute Inc., Cary, NC, 1989–2023).

3. Results

3.1. Pre-treatment FEC and copro-culture

The mean FEC on day 0 exceeded the threshold of 150 epg on all farms (Table 1). There was a significant interaction of location and host species ($P = 0.049$), such that sheep had an initial FEC (day 0) of 1255 ± 320 and 1312 ± 394 epg in the Northern and Western divisions, respectively, and goats had an FEC of 358 ± 320 and 2036 ± 320 in the Northern and Western divisions, respectively. The Western Division had a higher overall FEC (sheep and goats combined) than the Northern region (1674 ± 254 and 806 ± 227 epg, respectively, $P = 0.039$). However, there was no significant difference ($P = 0.807$) between host species (sheep and goats) in FEC. The larval culture from both host species in both locations identified four GIN genera, namely *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., and *Strongyloides* spp., with the proportions of 3 genera cultured shown in Fig. 1. *Strongyloides* spp. infection was at low levels in both host species and

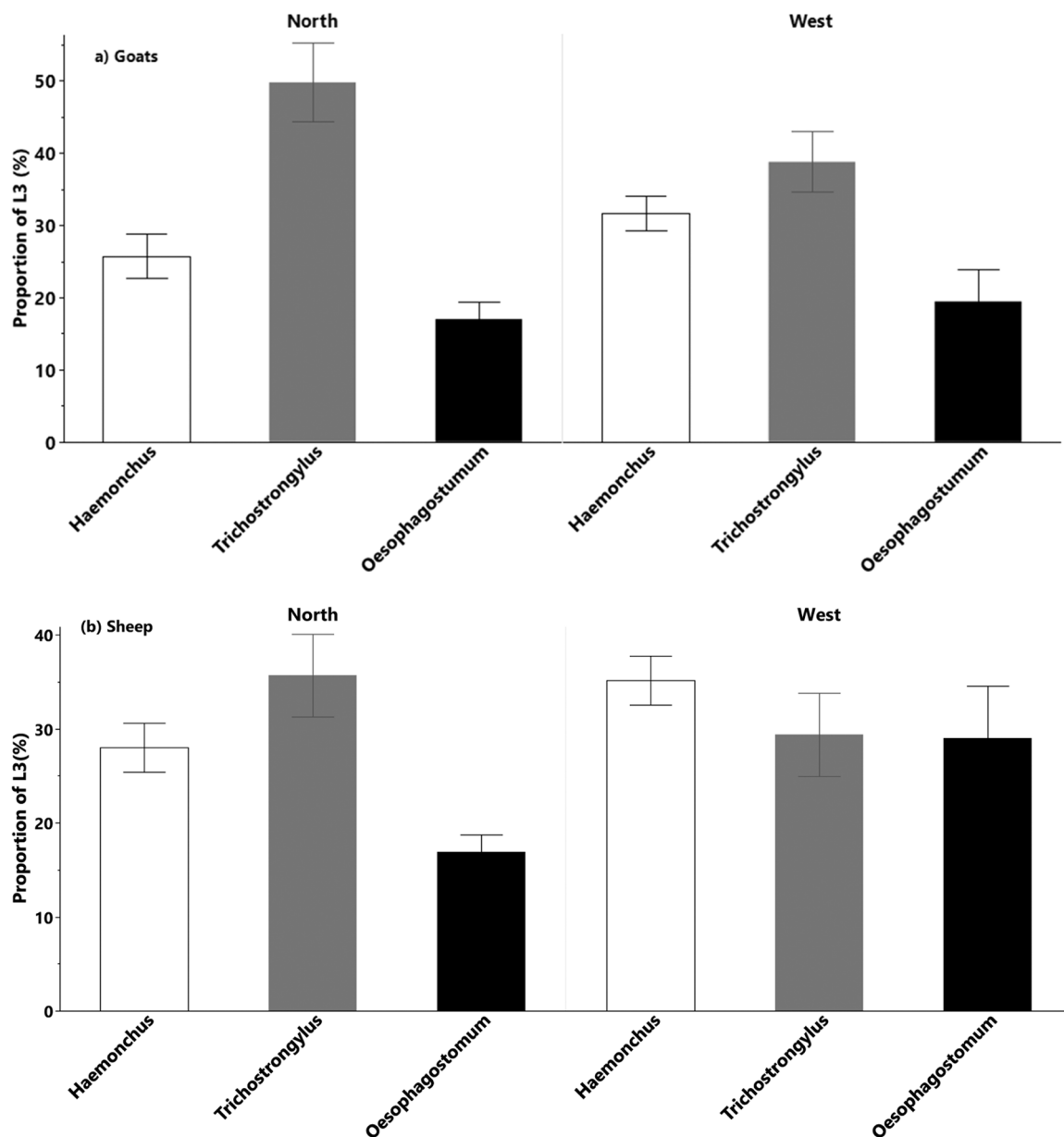


Fig. 1. Proportion of gastrointestinal nematode genera detected in copro-cultures obtained from a) Goat or b) Sheep faecal samples on day 0 in the Northern and Western divisions of Fiji. Error bars represent the standard error.

locations. The most commonly identified genera from the copro-cultures were *Trichostrongylus* spp., comprising 49 % and 39 % of the samples from goats in the Western and Northern divisions, and *Haemonchus* spp. (which predominated in sheep of the Western division only, accounting for 47 % of those samples, Fig. 1).

3.2. Change in control group FEC over time (days 0, 14, 28 and 42)

The untreated control groups' FEC mostly declined over time (on 81 % of farms), with only two farms (1 and 9) increasing between days 0 and 14. The pattern of declining FEC continued after day 14 in most of the control groups of the seven farms in which the persistence of the effects of MOX and CLO was tested after day 14 (Fig. 2).

3.3. Anthelmintic efficacy at day 14 post-treatment

Albendazole had the lowest overall average efficacy (FECR) of the anthelmintic treatments, followed by LEV, LEV+ALB, IVM and MOX (Fig. 3). Some small variation in treatment efficacy was observed across the different host species and farms (Table 3). Anthelmintic resistance was present on all 4 of the goat farms where ALB was tested (Table 3), as well as in 2 out of 3 sheep farms, while only 1 flock was classified as susceptible to ALB (Table 3). The results for LEV showed that 3 out of the 4 goat farms were classified as susceptible (Table 3). However, resistance was detected on 1 goat property (Table 3). Levamisole had a lower efficacy in sheep than in goat farms (Table 3). Gastrointestinal nematodes in 2 out of 3 sheep flocks were resistant to LEV, and 1 was classified as low resistance given the lower 95 % confidence interval (CI) being below 90 % (Table 3). As expected, the combination of LEV+ALB had higher efficacy than either active alone or active agents applied separately (Table 3). Worms in all 4 goat herds were found to be susceptible to LEV+ALB (Table 3). However, 1 sheep flock showed resistance to the combination treatment; the same flock was found resistant to LEV, but not resistant to ALB when applied individually. The other 2 sheep flocks were classified as low resistance and susceptible to the combination treatment. Resistance to IVM was detected only in 1 goat herd, while the other 3 were susceptible to this drug (Table 3). One of the sheep flocks was resistant to IVM; however, it presented as low resistance, and the other 2 were susceptible (Table 3). Moxidectin was the treatment that presented the highest efficacy, with no goat or sheep flock exhibiting resistance to this drug (Table 3). Nevertheless, 2 goat herds were classified as having low resistance to MOX, given the lower 95 % CI below 90 % (Table 3). Closantel had lower overall efficacy in sheep than in goat farms (Table 4).

Anthelmintic efficacy at day 14 post-treatment, differentiated by GIN genus, is shown in Table 4. Levamisole was broadly effective against all genera with reduced efficacy against *Haemonchus* spp., particularly in sheep. Albendazole showed reduced efficacy against both *Haemonchus* spp. and *Trichostrongylus* spp. in sheep and goats, respectively, while retaining high efficacy against *Oesophagostomum* spp. and *Strongyloides* spp. (Table 4). The combination of LEV+ALB had a mean efficacy of 99 % against all four GIN species in goats. At the same time, effectiveness varied on sheep farms, showing reduced efficacy against *Haemonchus* spp. and *Trichostrongylus* spp. on some farms (Table 4). Both IVM and MOX were effective against all genera on goat and sheep farms. Closantel had 100 % FECR against *Haemonchus* spp. on all sheep farms, but efficacy was below 95 % on 2 goat farms (Table 4).

3.4. Anthelmintic efficacy at days 28 and 42 following treatment with moxidectin and closantel

Moxidectin demonstrated overall FECR of 81 % and 74 % on days 28 and 42, respectively with considerable variation between farms and nematode genera (Tables 5 and 6). Efficacy tended to be higher against *Oesophagostomum* spp. and *Strongyloides* spp. than *Haemonchus* spp. and *Trichostrongylus* spp. Closantel showed 99–100 % effectiveness against *Haemonchus* on all farms on day 28 (Table 5), but efficacy was reduced at day 42 post-treatment, with only 2 of 6 farms having an efficacy of 95 % or greater (Table 6).

4. Discussion

This study aimed to update the knowledge on the anthelmintic resistance status of small ruminant farms in Fiji. While the study's main aim has been achieved, the limitations of this research must be acknowledged for the interpretation of the results. Small ruminant systems in Fiji are characterised by small properties with limited infrastructure and management practices, making it difficult to implement farm trials. The study has aimed for a minimum of 10 animals in each group; however, due to a limited number of animals on the farm or animals missing before the last sample collection, the final group was often less than 10, with an average of 7.2 animals across all groups. This is particularly important if a contemporaneous untreated control group is used to determine the reduction in FEC at any given time point, as used in this study. The smaller number of animals could contribute to the variability of results observed across farms. Similarly, the study aimed to identify a minimum of 100 L3 larvae per sample to analyse general distribution. However, this number was rarely matched on post-

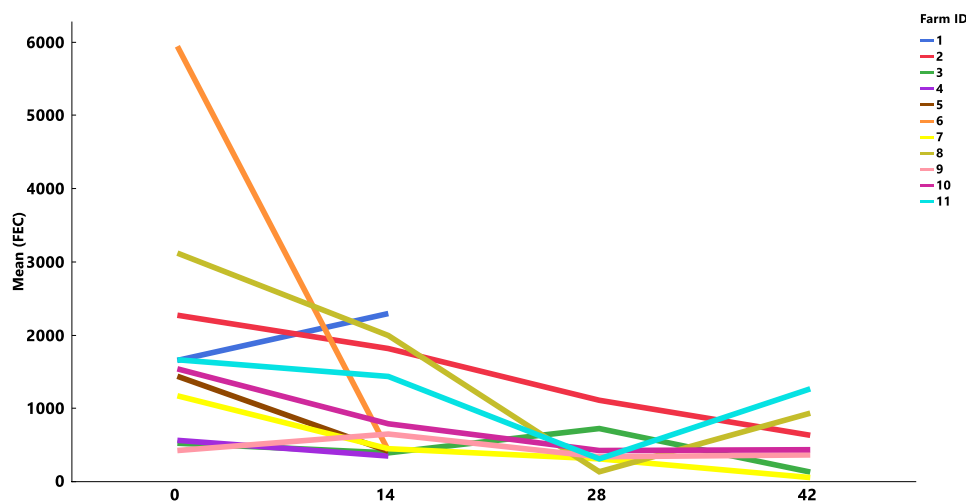


Fig. 2. Distribution of mean faecal egg counts (FEC) of the control group on days 0, 14, 28 and 42 (over time) on 11 farms located in the Northern and Western Divisions of Fiji.

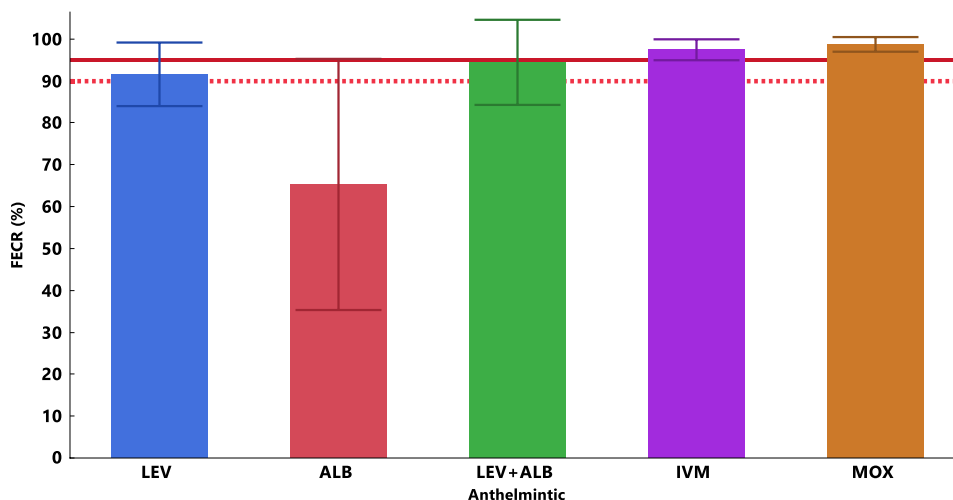


Fig. 3. Mean faecal egg count reduction (FECR) of anthelmintic treatments administered to sheep and goats in Fiji's Western and Northern divisions. Means were calculated across all farms ($n = 11$) and host species ($n = 2$) included in the study. Error bars represent the confidence intervals. Solid and dashed lines indicate 95 % and 90 % FECR, respectively. Levamisole (LEV); Albendazole (ALB); Levamisole and Albendazole (LEV+ALB); Ivermectin (IVM); Moxidectin (MOX).

treatment samples, given the very low presence of eggs in samples, which increases the level of uncertainty when calculating the FECR for the specific GIN genera. Taking all considerations, the study tested a significant number of farms ($n = 11$) and small ruminants ($n = 443$) animals under a realistic scenario, which reinforces the applicability of the results presented in this work.

The FECR tests were conducted at different points in time most farms in the Western division tested between February and April (Wet season), whereas in the Northern division was from May to July (Dry season). The different seasons when the FECR tests were conducted are possibly the reasons for the difference in FEC observed between the Western and Northern Divisions (1674 vs 806 epg). Nevertheless, mean pre-treatment FEC levels in both locations were well above the minimum recommendations for FECR, i.e., 150–200 epg for *Trichostrongylus* spp. and 500 epg for *Haemonchus* spp. (Coles et al., 2006), ensuring good power to detect reductions in FEC. Due to the limited group size, the results of this study are best interpreted at a broad level (e.g. mean efficacy values) rather than focusing on the results of individual groups or farms. The small flock and herd sizes (characteristic of the local production systems) of Fijian farms presented some constraints to the design of this study, including the ability to test all treatments simultaneously on all farms. Nevertheless, testing the treatments in farms that represented the characteristics of the region was necessary for the applicability of results, so the treatments were divided into three groups, and each treatment was tested in 7 different farms.

The mean efficacy in reducing FEC 14 days post-treatment exceeded 95 % for IVM and MOX and fell below that threshold for LEV, ALB and LEV + ALB (Fig. 3). In this study, 6 of 7 farms (87.5 %) exhibited resistance to ALB, and 3 out of 7 farms were resistant to LEV (42 %), compared with 44 % and 38 %, respectively, in 1986 (Banks et al., 1987). However, the previously mentioned work was published in grey literature and offered limited information on the analyses conducted and does not mention the mean FECR value for ALB across all farms. Therefore, it's not known if the mean efficacy level for this anthelmintic has changed but given the rise in the proportion of farms where AR was detected, it's likely that the mean FECR for ALB was higher in the past. The proportion of farms where resistance (i.e. FECR < 95 %) to ALB was detected in 1986 was 44 %, whereas in this study, it increased to 86 % (Banks et al., 1987). The low efficacy of the registered ALB dose may be due to resistance or inadequacy of the registered dose since there are indications that the bioavailability of ALB is lower in goats, which led some authors to recommend a higher dosage than sheep (Hennessy,

1994). ALB is one of the few anthelmintic drugs which is registered for sheep and goats in Australia, and the dosage used in this study followed the manufacturer's recommendation of 3.8 mg/kg of LW. Contrary to ALB, the proportion of farms where resistance was observed did not appear to have changed much since the reports from 1986 (38 % in 1986 and 43 % now). The possible reasons for these differences over time are unclear, given that ALB has not been commercialized in the country over a few years (~ 5 years), and LEV has been the only option available to farmers over recent years. However, detailed records of anthelmintic approval and importation over the period are unavailable, and the period of utilization of these products is reported by local farmers and ministry staff. A key factor is likely to be the presence of refugia in pastures and untreated animals. Parasites in refugia are not exposed to anthelmintic at the time of treatment, causing the treatment to be a weak selection event across the parasite population (Van Wyk, 2001). Under management systems in Fiji, refugia occurs when the traditional cost-saving practice of only treating clinically affected animals is practised or when animals are grazed or tethered on common land shared with non-treated animals. However, why this would prevent the increase of resistance to LEV but not ALB is not clear. Other possible reasons could be low drench frequency due to the availability of anthelmintics, which are generally only commercialized by the Government Veterinary Clinics, which have limited stock and frequently suffer disruptions in supply.

The most common GIN genera encountered in copro-cultures before and after anthelmintic treatments were *Trichostrongylus* spp. and *Haemonchus* spp. The importance of *Trichostrongylus* spp. as one of the main GIN parasites in Fijian SR farms has been previously reported by Manueli (2004). While *Haemonchus* spp. is often the most prevalent and pathogenic species in the humid tropics, the marked dry season experienced in the Western and Northern divisions of Fiji where the study was carried out, would not favour *Haemonchus* spp., as the eggs of this species are very susceptible to desiccation and have a very limited time to hatch (5 days) (Barger et al., 1972; OConnor et al., 2006). In the current study, populations of *Haemonchus* spp. and *Trichostrongylus* spp. resistant to ALB, LEV and IVM were observed in specific locations without any distinct pattern of occurrence. Uniformly high efficacy of most anthelmintics tested (except CLO) was observed against *Oesophagostomum* spp. and *Strongyloides* spp. *Strongyloides* spp. has been considered less pathogenic in the past compared to other nematodes. However, a recent study reported it as highly pathogenic and responsible for the death of young animals during an outbreak in Uruguay

Table 3

Faecal egg count reduction (FECR%) and anthelmintic resistance status observed at day 14 (post-treatment) for a range of anthelmintic treatments (LEV, ALB, LEV+ALB, IVM, MOX and CLO) on private sheep and goat farms in Fiji.

Anthelmintic	Species	Location	Animals(n)	Farm	Day 14 FEC (EPG)	^a FECR(%)	Upper 95 % CL	Lower 95 % CL	Resistance status
Control	Goat	West	10	1	2295	-	-	-	-
		West	9	2	1817	-	-	-	-
		West	9	3	393	-	-	-	-
		North	7	4	350	-	-	-	-
		North	9	5	416	-	-	-	-
		North	6	6	425	-	-	-	-
	Sheep	West	8	7	450	-	-	-	-
		West	10	8	1995	-	-	-	-
		North	6	9	650	-	-	-	-
		North	8	10	790	-	-	-	-
		North	7	11	1436	-	-	-	-
		North	7	11	1436	-	-	-	-
LEV	Goat	West	9	1	61	97	99	92	Susceptible
		West	10	2	80	95	98	91	Susceptible
		North	8	3	75	81	96	-1	Resistant
		North	7	4	0	100	100	95	Susceptible
	Sheep	West	8	5	88	81	94	44	Resistant
		North	7	6	50	88	97	55	Resistant
		North	8	7	0	99	100	87	Low Resistant
		North	8	7	0	99	100	87	Low Resistant
ALB	Goat	West	8	1	567	72	96	-85	Resistant
		West	9	2	767	58	83	-2	Resistant
		North	7	3	193	51	90	-152	Resistant
		North	8	4	56	84	96	31	Resistant
	Sheep	West	8	5	0	100	100	96	Susceptible
		North	8	6	50	89	97	60	Resistant
		North	7	7	186	3	76	-297	Resistant
		North	7	7	186	3	76	-297	Resistant
LEV+ALB	Goat	West	9	1	11	100	100	96	Susceptible
		West	10	2	90	95	97	91	Susceptible
		North	7	3	0	100	100	95	Susceptible
		North	7	4	0	100	100	95	Susceptible
	Sheep	West	7	5	125	70	90	3	Resistant
		North	7	6	0	100	100	96	Susceptible
		North	9	7	2	96	99	81	Low Resistant
		North	9	7	2	96	99	81	Low Resistant
IVM	Goat	West	10	2	80	96	98	91	Susceptible
		West	9	8	50	97	99	90	Susceptible
		North	8	3	0	100	100	96	Susceptible
		North	10	9	45	93	99	54	Resistant
	Sheep	West	10	10	30	96	99	88	Low Resistant
		North	9	7	0	100	100	97	Susceptible
		North	7	11	0	100	100	92	Susceptible
		North	7	11	0	100	100	92	Susceptible
MOX	Goat	West	10	2	65	96	99	88	Low Resistant
		West	9	8	75	96	100	71	Low Resistant
		North	10	9	0	100	100	98	Susceptible
		North	7	3	0	100	100	95	Susceptible
	Sheep	West	10	10	50	99	100	94	Susceptible
		North	9	7	0	100	100	97	Susceptible
		North	8	11	0	100	100	93	Susceptible
		North	8	11	0	100	100	93	Susceptible
CLO	Goat	West	10	2	366	80	92	52	N/A*
		West	10	8	1655	25	75	-125	N/A*
		North	10	9	150	77	95	2	N/A*
		North	7	3	186	53	90	-117	N/A*
	Sheep	West	9	10	130	82	93	50	N/A*
		North	7	7	278	38	85	-151	N/A*
		North	7	11	436	0	78	-348	N/A*
		North	7	11	436	0	78	-348	N/A*

Levamisole (LEV); Albendazole (ALB); Levamisole and Albendazole (LEV+ALB); Ivermectin (IVM); Moxidectin (MOX); Closantel (CLO); Low resistant - when FECRT and upper confidence level is equal or above 95 % but lower confidence level is below 90 %. * Not applicable because CLOS has only a narrow spectrum efficacy claim and no values for the Control group (FECRT%, Upper CL, Lower CL and response); a FECR% was calculated relative to the treatment/control group. When encountering a negative FECR%, the value is truncated to 0. Instead of using "suspected resistance," the term "low resistance" is used, which is considered a subclassification of the resistant category by [Kaplan et al. \(2023\)](#).

([Romero et al., 2022](#)).

Gastrointestinal nematode populations were susceptible to LEV+ALB combination in 6 of 7 farms in the current study. This can be attributed to the independent effect of each drug rather than any synergism between their effects ([Anderson et al., 1991](#)). Using the combination of anthelmintics also allows for increasing the effectiveness of the individual actives, even if they are less potent when used separately ([Le Jambre et al., 2010](#)). The combination of drugs with different action mechanisms slows down the development of resistance, as resistance to the combination requires individual parasites to carry two separate sets of resistant alleles which has a lower probability of occurrence

([McKenna et al., 1996](#)). Ivermectin and MOX belong to the macrocyclic lactone (ML) group of anthelmintics. Moxidectin was not previously used in smallholder and semi-commercial farms in Fiji and as expected, no resistance was detected for this drug except low resistance on two goat farms. Both drugs, IVM and MOX, exhibited mean efficacy higher than 95 % at 14 days post-treatment. However, resistance to IVM was observed in one goat farm where the efficacy was 93 %. These results are broadly consistent with those of [Banks et al. \(1987\)](#) who found no resistance to IVM on 9 commercial farms where it was tested. The oral anthelmintic MOX effectively kept the *Haemonchus* spp. FEC at zero in all sheep and on 50 % of goat farms until at least day 28. The label claims

Table 4
Anthelmintic efficacy against four GIN genera at day 14 by host species and farm.

Anthelmintic	Species	Number of farms	Number of animals tested	Overall and individual farm FECR (%)	Larval reduction values and values for each farm in brackets			
					<i>Haem</i>	<i>Trich</i>	<i>Oeso</i>	<i>Stro</i>
LEV	Goat	4	34	93 % (81, 95, 97, 100)	92 % (77, 94, 97, 100)	95 % (78, 100, 100, 100)	95 % (79, 100, 100, 100)	100 % (100, 100, 100, 100)
	Sheep	3	23	86 % (81, 88, 89)	77 % (48, 83, 99)	95 % (87, 97, 100)	97 % (90, 100, 100)	100 % (100, 100, 100)
ALB	Goat	4	32	66 % (51, 58, 72, 84)	71 % (12, 73, 100, 100)	41 % (0, 21, 54, 87)	98 % (95, 95, 100, 100)	94 % (77, 100, 100, 100)
	Sheep	3	23	64 % (3, 89, 100)	63 % (0, 88, 100)	60 % (0, 79, 100)	92 % (100, 100, 76)	100 % (100, 100, 100)
LEV+ALB	Goat	4	33	99 % (95, 100, 100, 100)	98.5 % (94, 100, 100, 100)	99 % (97, 100, 100, 100)	98.7 % (95, 100, 100, 100)	98 % (92, 100, 100, 100)
	Sheep	3	23	89 % (70, 96, 100)	92 % (79, 96, 100)	80 % (47, 93, 100)	100 % (100, 100, 100)	100 % (100, 100, 100)
IVM	Goat	4	37	96.5 % (93, 96, 97, 100)	98 % (93, 97, 100, 100)	95 % (89, 93, 96, 100)	99 % (95, 100, 100, 100)	100 % (100, 100, 100, 100)
	Sheep	3	26	99 % (96, 100, 100)	96 % (89, 100, 100)	100 % (100, 100, 100)	100 % (100, 100, 100)	100 % (100, 100, 100)
MOX	Goat	4	36	98 % (96, 96, 100, 100)	94 % (87, 88, 100, 100)	100 % (100, 100, 100, 100)	100 % (100, 100, 100, 100)	100 % (100, 100, 100, 100)
	Sheep	3	26	99.6 % (99, 100, 100)	96 % (89, 100, 100)	100 % (100, 100, 100)	100 % (100, 100, 100)	100 % (100, 100, 100)
CLO	Goat	4	36	59 % (25, 53, 77, 80)	93 % (80, 93, 100, 100)	35 % (0, 19, 53, 66)	71 % (0, 84, 100, 100)	75 % (0, 100, 100, 100)
	Sheep	3	23	40 % (0, 38, 82)	100 % (100, 100, 100)	4 % (0, 0, 13)	31 % (0, 0, 93)	100 % (87, 100, 100)

Haemonchus (*Haem*); *Trichostrongylus* (*Trich*); *Oesophagostomum* (*Oeso*); *Strongyloides* (*Stro*); Levamisole (LEV); Albendazole (ALB); Levamisole and Albendazole (LEV+ALB); Ivermectin (IVM); Moxidectin (MOX); Clo-santel (CLO). Negative FECR% were truncated to a value of zero. Values in brackets represent the mean FECR on individual farms.

Table 5

Efficacy of moxidectin (MOX) and closantel (CLO) against four GIN genera on day 28 post-treatment on Fiji private sheep and goat farms.

Anthelmintic	Species	Number of farms	Number of animals tested	Overall and individual farm FECR (%)	Larval reduction values and values for each farm in brackets			
					<i>Haem</i>	<i>Trich</i>	<i>Oeso</i>	<i>Stro</i>
MOX	Goat	4	36	86 % (77, 83, 88, 96)	75 % (11, 87, 100, 100)	84 % (60, 87, 94, 96)	87 % (49, 100, 100, 100)	89 % (66, 89, 100, 100)
	Sheep	3	28	75 % (68, 68, 89)	100 % (100, 100, 100)	76 % (64, 64, 100)	100 % (100, 100, 100)	100 % (100, 100, 100)
CLO	Goat	4	37	57.5 % (0, 63, 80, 87)	100 % (99, 100, 100, 100)	47 % (0, 50, 55, 81)	93 % (79, 91, 100, 100)	100 % (100, 100, 100, 100)
	Sheep	3	28	42 % (32, 32, 62)	100 % (100, 100, 100)	29 % (25, 25, 37)	100 % (100, 100, 100)	57 % (32, 32, 100)

Haemonchus (*Haem*); *Trichostrongylus* (*Trich*); *Oesophagostomum* (*Oeso*); *Strongyloides* (*Stro*), Moxidectin (MOX); Closantel (CLO). Values in brackets represent the mean FECR on individual farms.

Table 6

Efficacy of moxidectin (MOX) and closantel (CLO) against four GIN genera on day 42 post-treatment on Fiji private sheep and goat farms.

Anthelmintic	Species	Number of farms	Number of animals tested	Overall and individual farm FECR (%)	Larval reduction values and values for each farm in bracket			
					<i>Haem</i>	<i>Trich</i>	<i>Oeso</i>	<i>Stro</i>
MOX	Goat	4	36	67.5 % (0, 79, 95, 96)	42.5 % (0, 0, 70, 100)	68 % (0, 72, 99, 100)	99.5 % (98, 100, 100, 100)	72 % (0, 88, 100, 100)
	Sheep	3	27	83 % (78, 91, 96)	93 % (80, 100, 100)	79 % (69, 71, 97)	89 % (80, 88, 100)	100 % (99, 100, 100)
CLO	Goat	34	36	35 % (5, 29, 33, 73)	56 % (11, 38, 79, 95)	39 % (0, 28, 43, 83)	27 % (0, 0, 49, 58)	88 % (80, 81, 90, 100)
	Sheep	3	26	39 % (0, 34, 84)	79 % (44, 94, 100)	32 % (0, 0, 97)	92 % (82, 94, 100)	71 % (18, 96, 100)

Haemonchus, (*Haem*); *Trichostrongylus* (*Trich*); *Oesophagostomum* (*Oeso*); *Strongyloides* (*Stro*); Moxidectin (MOX); Closantel (CLO). Values in brackets represent the mean FECR on individual farms.

oral MOX prevents reinfection from *Haemonchus* worms in sheep for at least 14 days and prevents pasture contamination from *Haemonchus* spp. eggs for at least 30 days after a single treatment. However, there is no label claim for persistent activity against *Trichostrongylus* spp., and this genus reappeared on sheep and goat farms by day 28. Additionally, there was some evidence of a sustained reduction in FEC relative to controls on day 42 post-treatment. In other studies, FECR above 95 % was observed for up to 77 days in sheep when applied topically, where authors justified this effect due to the persistent binding of this molecule with plasma proteins for at least 60 days (Rana et al., 2001). In the present study design, the apparent effects at day 42 post-treatment cannot be separated from a short-term effect of removing worm burdens and thus reducing future FEC relative to untreated controls, thereby weakening the infection-impacting control groups. Multiple factors could have contributed to the reduced FEC observed in the control groups. Untreated groups may have lower FEC due to the host's natural immune response, which can control worm populations over time. Environmental factors like less favourable conditions for GIN survival can also play a role. The reduction of GIN infection in treated animals could have led to a dilution of infection across the overall farm (Van Wyk et al., 2006), which in turn resulted in decreased FEC in the untreated groups.

Closantel demonstrated low overall efficacy at day 14 post-treatment on both sheep and goat farms. This result could be expected as it is a narrow-spectrum anthelmintic that is used to target specifically *Haemonchus contortus*. It was effective against *Haemonchus* spp. in both host species, although full efficacy at reducing FEC (≥ 95 % FECR) was only evident at 28 days post-treatment. On day 42, post-treatment FECR was below 95 % on 4 of 6 farms tested, which is inconsistent with the label claim of prevention of reinfestation with *Haemonchus* spp. for 4 weeks after dosing.

5. Conclusion

In Fiji, the prevalence of anthelmintic resistance appears to have

slowly increased for ALB in nearly 4 decades. On the other hand, satisfactory efficacy was observed for anthelmintics or combinations not previously introduced to private farms in Fiji. Overall, the results show that the AR level is lower than this study hypothesised, especially for LEV. A key recommendation would be not to utilise ALB as a single active. As the registered dose of ALB has been found less efficacious in the present study and elsewhere, another recommendation would be to research the potential to use a higher dose (e.g. 7.5 mg/kg) than the registered dose used in this study in both host species for ALB. While MOX and IVM will likely effectively control GIN on Fijian farms, alternative protocols for using the currently approved ALB and LEV are more attractive than introducing a new and relatively more expensive anthelmintic active such as MOX. The LEV+ALB combination was found efficacious and offers an attractive treatment option for Fiji. Restricting the use of ML anthelmintics on farms in Fiji would continue to restrict the development of resistance to this important class of anthelmintics. There is little rationale for introducing CLO at this point, as *Haemonchus* spp. (for which CLO is a narrow-spectrum treatment) forms a minority of L3 larvae on the tested farms. More frequent monitoring (2–3 years) of the status of anthelmintic efficacy will be important to assess the effect of any changes made to the importation and/or commercialization of anthelmintic drugs in the country.

CRedit authorship contribution statement

Kour Gurdeep: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Carvalho da Silva Tiago Alves Correa:** Writing – review & editing, Resources, Project administration, Investigation, Conceptualization. **Walkden-Brown Stephen W.:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Formal analysis, Conceptualization. **Cowley Frances C.:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Baleiverata Alice:** Methodology. **Mala Shayna:** Methodology. **Rao Ritesh:** Methodology. **Prasad Divesh:**

Methodology.

Declaration of Competing Interest

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