RECyT
Year 27 / Nº 44 / 2025 /
DOI: https://doi.org/10.36995/j.recyt.2025.44.004

Gastrointestinal nematodes in small ruminants: genus identification and evaluation of anthelmintic resistance in Misiones, Argentina

Nematodos gastrointestinales en pequeños rumiantes: identificación de géneros y evaluación de resistencia antihelmíntica en Misiones, Argentina

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Received: 04/11/2024; Accepted: 24/07/2025

Abstract
Infections by gastrointestinal nematodes (GIN) are a significant cause of productivity losses in sheep and goat farming. Anthelmintic resistance is a growing issue, driven by the indiscriminate use of these products. This study examines the GINs in sheep and goats in the province of Misiones, Argentina, and the resistance to anthelmintic drugs. Ten farms from southern Misiones were analyzed, with fecal egg counts (FEC) and drug resistance tests were performed. A wide variability in parasite burden was observed both between and within herds, highlighting the importance of customizing treatments. The average FEC was 309 and was established as the threshold above which an individual should be treated. Strategic treatment is proposed, targeting animals with high parasite loads, considered "spreaders" of infection. This approach optimizes the use of anthelmintics, reducing selection pressure on the parasite population and helping prevent resistance. Resistance to ivermectin was detected in all evaluated farms, as well as to benzimidasole and closantel, confirming the need for laboratory diagnostics before administering treatments. The predominant GIN genus was *Haemonchus*, represented by *H. contortus* and *H. placei*, followed by *Cooperia.* Additionally, a Polymerase Chain Reaction (PCR) technique was adapted for the rapid detection of the most common genera, which will improve parasite diagnosis and characterization. This study describes the GIN and anthelmintic resistance in sheep and goats in the province of Misiones, and underscores the need for continued research to adapt control strategies to local conditions and ensure the sustainability of production.

Keywords: Gastrointestinal parasites, *Haemonchus*, FEC, Anthelmintic resistance, Strategic control.

Resumen
Las parasitosis por nematodos gastrointestinales (NGI) son una causa significativa de pérdidas productivas en la ganadería ovina y caprina. La resistencia a los antihelmínticos es un problema creciente, impulsada por el uso indiscriminado de estos productos. Este trabajo estudia a los NGI en ovinos y caprinos de la provincia de Misiones, Argentina, y la resistencia a las drogas antihelmínticas. Se analizaron 10 establecimientos del sur de Misiones, obteniendo valores de huevos por gramo (HPG) y realizando test de resistencia a drogas. Se observó una amplia variabilidad de la carga de parásitos entre y dentro de cada rebaño, destacando la importancia de personalizar los tratamientos. El valor promedio del HPG fue de 309, estableciéndose como umbral a partir del cual un individuo debe ser tratado. Se propone el tratamiento estratégico, dirigido a aquellos animales con cargas parasitarias elevadas, considerados como "diseminadores" de la infección. Esto permite optimizar el uso de antihelmínticos, reduciendo la presión de selección sobre la población de parásitos y contribuyendo a evitar la resistencia. Se detectó entre un 90,3% y 35% de resistencia a la ivermectina en los dos campos evaluados, así como un 60,7% y 32,2% al febendazol y 25,9% al closantel, lo que confirma la necesidad de implementar diagnósticos de laboratorio antes de proceder con tratamientos. El género de NGI predominante fue *Haemonchus*, representado por *H. contortus y H. placei*, seguido por *Cooperia*. Además, se puso a punto una técnica de Reacción en Cadena de la Polimerasa para la detección rápida de los géneros más comunes, lo que permitirá mejorar el diagnóstico y caracterización de los parásitos. Este trabajo describe los NGI y la resistencia a los antihelmínticos en ovinos y caprinos en la provincia de Misiones, y destaca la necesidad de continuar realizando investigaciones para adaptar las estrategias de control a las particularidades locales y garantizar la sustentabilidad de la producción.

Palabras clave: Parásitos gastrointestinales, *Haemonchus*, HPG, Resistencia antihelmíntica, Cabras, Ovejas.

INTRODUCTION

Gastrointestinal nematodiasis, or verminous gastroenteritis, is a multifactorial disease caused by gastrointestinal nematodes (GIN) of various genera and species that affect ruminants in different segments of their digestive tract [1]. The disease is generally subclinical and constitutes an important cause of production losses [2]. These infections trigger a series of harmful signs such as weakness, depression, intestinal inflammation, diarrhea, edema, general debilitation, hemorrhages in the gastrointestinal tract, and metabolic alterations in protein processing, leading to reduced weight gain and, in severe cases, death [3]. Infection with GIN has direct effects on weight gain, body development, reproductive performance, and milk production, as well as indirect effects such as underutilization of forage resources and increased susceptibility to diseases. In addition, the costs associated with veterinary treatments contribute to higher production expenses, ultimately reducing profitability [4], [5].

GIN are considered endemic and do not have significant regulatory or commercial implications; therefore, their control remains largely the responsibility of farmers and/or veterinarians [6]. The main GIN species affecting sheep and goats include *Haemonchus spp., Cooperia spp., Teladorsagia (Ostertagia) circumcincta, Trichostrongylus spp., Nematodirus spp., and Oesophagostomum spp.*, which may be located in different segments of the ruminant digestive tract [7].

Diagnosis of GIN parasitism is performed through a fecal egg count (FEC), which is an indicator of the parasitic load carried by the animal [8].

Control of gastrointestinal parasitoses is achieved through the use of anthelmintic drugs and parasite management strategies, which encompass all grazing system measures aimed at reducing pasture contamination with eggs or L3 larvae infestations [9], [10]. Benzimidazoles (albendazole and fenbendazole), imidazothiazoles (levamisole), salicylanilides (closantel), and macrocyclic lactones (ivermectin) are broadly employed nematicidal compounds commonly used in ruminants both in the province of Misiones and throughout Argentina [11]. Anthelmintic drugs have recommended dosages according to their pharmacological use in cattle and sheep. However, in goats, the absorption of anthelmintics is lower than in other ruminants, and they are metabolized and excreted more rapidly; therefore, the recommended dose is twice that used for sheep [12]. It is well known that the use of anthelmintic drugs favors the multiplication of resistant individuals, representing a threat to the livestock industry [9], [10]. GIN have genetic characteristics that promote the rapid development of resistance to different drugs (10). At present, resistance has been detected to all drugs available on the market, including those of the latest generation. Worldwide [13]–[15], as well as in our country and in the NEA region (Northeast Argentina) [7], [11], [12], [16]–[21], the implementation of appropriate control strategies and treatments based on laboratory analyses has become both a necessity and an active field of research [17], [22].

In Argentina, studies on GIN have been conducted in several provinces, with the exception of Misiones [2], [4], [28], [11], [12], [20], [23]–[27]. Misiones has a production system based on smallholdings, where most producers operate small farms that are often not self-sustaining [29]. This situation encourages the formation of associations, giving rise to production basins, among which the Sheep and Goat Production Basin (COC) of Southern Misiones serves as the prime example [30]. This basin encompasses more than 25,000 head of sheep and goats distributed among approximately 450 producers from the municipalities of Candelaria, Garupá, Cerro Corá, Cerro Azul, Santa Ana, Fachinal, Profundidad, Olegario Víctor Andrade, and San José [31]. Although preliminary studies on GIN parasitoses have been conducted [18], [32], their impact on local herds remains unknown.

Therefore, the aim of this study was to investigate gastrointestinal nematodes in sheep and goats from Misiones, to evaluate resistance to anthelmintic drugs (ivermectin, closantel, fenbendazole, and levamisole), and to establish a molecular biology assay for genus characterization. The research focused on fecal egg count (FEC) analysis in farms within the COC, with the objective of generating key information to improve parasite control in the region.

MATERIALS AND METHODS

Experimental design

Ten farms from the Sheep and Goat Production Basin (COC) in southern Misiones were selected based on their proximity and/or at the owner's request (convenience sampling) [33], during September 2022 and March 2023 (Figure 1). A management survey was conducted at each farm to determine management conditions. Fecal samples (FS) from sheep and goats were collected to evaluate the number of GINs through fecal egg counts. In addition, fecal samples were pooled by farm to perform coprocultures and identify nematode genera under an optical microscope. From each pool, a sample was also processed to determine the genera present by PCR. After establishing the average FEC threshold, resistance testing was performed on farms that exceeded this value.

Sample collection
Fecal samples were collected directly from the rectum of adult animals (both females and males) showing no signs of disease, using clean, labeled polyethylene bags, and stored in insulated containers for transport. As an inclusion criterion, animals must not have received anthelmintic treatment during the three months prior to sampling.

Fecal Egg Counts
The fecal egg count (FEC) was performed using the modified INTA McMaster chamber technique [2]. This method is based on the flotation principle, in which the eggs present in a fecal sample—when mixed with a supersaturated sodium chloride (NaCl) solution—separate from the fecal mass and rise to the surface of the aqueous solution [2]. A supersaturated (SS) NaCl solution was prepared, and feces were mixed at a 1:20 (w/v) ratio, corresponding to 2.5 g of feces in 50 ml of SS NaCl. The mixture was vortexed until the feces were completely disaggregated, then left to stand for 5 minutes. A sample from the upper portion of the supernatant was collected and loaded into the McMaster chamber, and the fields were then counted. The resulting value was multiplied by the dilution factor (20), thereby obtaining the helminth fecal egg count (FEC).

Coproculture
Coprocultures were performed for the identification of L3 larvae in a set of samples [2]. From farms with an average FEC above the threshold and sufficient sample volume, pooled fecal material was collected in a container, and granulated Styrofoam® was added until a light, crumbly consistency was achieved. The coprocultures were prepared in plastic cups following the recommendations of Fiel et al. (2011) [2]. The cultures were incubated for 21 days at room temperature (20–28 °C), ensuring that moisture was maintained. After incubation, the culture was transferred to a conical beaker, submerged in chlorine-free warm water, and allowed to settle at room temperature for 12–24 hours. Infective larvae were recovered from the bottom of the conical beaker, and identification was performed under an optical microscope after immobilizing the samples with Lugol's solution. Identification was carried out based on morphological characteristics [2]. A total of 300 L3 larvae from each of the four fecal sample batches were counted, resulting in 1,200 infective L3 GIN larvae in total.

Genus Identification by Polymerase Chain Reaction (PCR)

Analytical samples
For the extraction of DNA from GIN eggs, 50 mL of the processed supernatant from pooled fecal samples were used. The material was distributed into three 15 mL tubes and centrifuged for 5 minutes at medium speed (first cycle). The supernatant was retained (discarding the pellet) and examined under an optical microscope to confirm the presence of parasite eggs. The procedure was then repeated (second cycle). During the third cycle, the supernatant was diluted 1:1 with distilled water and centrifuged for 5 minutes at medium speed. The supernatant was discarded, and the resulting pellets were combined into a single 1.5 mL microtube for subsequent DNA extraction.

DNA extraction
A volume of 200 µL of concentrated eggs and 600 µL of Isotonic Lysis Solution (ILS) were placed into a 1.5 mL microtube, homogenized by inversion three times, and incubated for 3 minutes. The sample was centrifuged at 6,500 ×g for 1 minute, and the supernatant was discarded. Then, 20 µL of Proteinase K solution and 150 µL of PBS were added, and the sample was vortexed for 1 minute to resuspend the pellet completely. Next, 200 µL of BL buffer were added and mixed in a vortex for 30 seconds. The mixture was incubated for 15–30 minutes at 56 °C. After incubation, 200 µL of ethanol (96%) were added, mixed by inversion three times, and briefly centrifuged again. The entire contents were then transferred onto a silica minicolumn placed over a 2 mL collection tube from the "ADN PuriPrep-T Kit" (Inbio Highway, Tandil, Argentina). DNA extraction was performed according to the manufacturer's instructions. The eluted DNA was stored at −20 °C.

PCR Reactions
PCR reactions were performed using the primers described by Zarlenga et al. (2001) [34], which amplify specific regions corresponding to five genera of GIN (Table 1). Each reaction was carried out in a final volume of 50 µL, containing 1X buffer, $MgCl_2$ (1.5 mM), 0.2 µM of each primer, 200 µM dNTPs, 1% dimethyl sulfoxide (DMSO), 1.25 µU of DNA polymerase, and 1 µL of target DNA. The cycling profile consisted of the following: 94 °C for 5 minutes, followed by 40 cycles at 94 °C for 1 minute, 60 °C for 1 minute, and at 72 °C for 2 minutes, with a final extension at 72 °C for 7 minutes.
Amplification products were separated on a 1.8% agarose gel, stained with ethidium bromide, and visualized under UV transillumination.

Table 1. Primers used for PCR.

Name	Target genus and species	Orientation	Amplicon size (pb)	Sequence orientation 5' → 3'
NGI-I	*Ostertagia ostertagi*	F-417	257	TAAAAGTCGTAACAAGGTATCTGTAGGT
		R-526		GTCTCAAGCTCAACCATAACCAACCATTGG
NGI-II	*Haemonchus placei*	F-628	176	CATTTTCGTCTTGGGCGATAT
		R-627		TGAGACCGCACGCGTTGATTCGAA
NGI-III	*Oesophagostomum radiatum*	F-595	329	GCAGAACCGTGACTATGGTC
		R-653		GACAAGGAGATCACGACATCAGCAT
NGI-IV	*Trichostrongylus colubriformis*	F-708	243	CAGGGTCAGTGTCGAATGGTCATTGTCAAATA
		R-707		CAGGGTCAGTGGTTGCAATACAAATGATAATT
NGI-V	*Cooperia oncophora*	F-618	151	TCGATGAAGAGTTTTCGGTGTTC
		R-641		TTCACGCTCGCTCGTGACTTCA

Information adapted from Zarlenga et al. (2001) [34].

Anthelmintic Resistance Test

In herds that exhibited a high average FEC value, an anthelmintic resistance test is (ART) was performed to determine which anthelmintic drug showed the greatest effectiveness.

In each herd, the test was conducted as follows: five groups of ten animals each, belonging to the same species, were randomly selected from within the herd (G1: Control, G2: Closantel, G3: Levamisole, G4: Ivermectin, and G5: Fenbendazole). Each group received only one drug, except for the control group, which received no treatment. The drugs were administered at the manufacturer's recommended dosage. Fecal samples were collected from each group before treatment (time 0) and 14 days post-treatment. For each sample, the FEC was determined; and the fecal egg count reduction (FECR) at 14 days was calculated using the formula recommended by Coles et al. (1992) (8):

FECR %: ((C – T) / C) x 100

Where T is the arithmetic mean of each treated group, and C is the arithmetic mean of the untreated control group 14 days post-treatment. In cases where an untreated control group could not be used, the reduction was calculated based on the initial (day 0) count, which served as the control reference [2].

RESULTADOS

Samples were collected from 10 farms located in the municipalities of Cerro Corá (N=4), Fachinal (N=1), and Profundidad (N=5), in the province of Misiones, Argentina (Figure 1).

Figure 1. Map of the province of Misiones showing its political division and highlighting the municipalities where samples were collected (gray). The upper left inset shows a map of Argentina indicating the location of the Misiones Province (green).

Survey results

Of the 10 farms sampled, 30% (3/10) were goat farms, and 70% (7/10) were sheep farms. Samples were collected from 36% (422/1177) of the animals. Of this total, 81% (343/422) corresponded to sheep and 19% (79/422) to goats. The mean Fecal Egg Count (FEC) was 309, while the mean of the maximum counts reached 2680. Except for Farm 1, non-parasitized animals (FEC = 0) were found on all farms (Table 2).

According to the survey responses related to deworming practices, only 10% (1/10) of producers had an established deworming plan, while 80% (8/10) reported deworming their animals at their convenience, with frequencies ranging from every 2 to 6 months. Regarding the

deworming strategy, in 80% (8/10) of the farms, the entire herd was treated with anthelmintics, while in 20% (2/10), treatment was administered only to animals showing clinical signs. For newly introduced animals, 60% (6/10) of the producers reported administering anthelmintics upon entry to the farm, and 40% (4/10) reported doing so to offspring at the time of weaning.

To calculate the anthelmintic dose, 60% of producers reported visually estimating the weight of each animal, whereas 30% used the heaviest animal in each category as a reference.

Among the farms analyzed, 30% (3/10) of producers reported using ivermectin in their most recent deworming, 20% (2/10) used closantel, 20% (2/10) levamisole, and 10% (1/10) fenbendazole, while 20% did not know or could not recall which antiparasitic drug had been used most recently (Table 3).

Table 2. Number of animals analyzed.

				FEC		
Farm ID	**N**	**n**	**%**	**$\bar{x}$**	**Max.**	**Min.**
Farm 1*	150	22	15%	450	1500	60
Farm 2*	17	17	100%	119	520	0
Farm 3^{s}	150	32	21%	266	1840	0
Farm 4*	280	40	14%	244	1920	0
Farm 5^{s}	108	60	56%	94	1440	0
Farm 6^{s}	42	42	100%	202	3260	0
Farm 7^{s}	60	43	72%	235	1340	0
Farm 8^{s}	75	69	92%	35	280	0
Farm 9^{s}	200	54	27%	749	8600	0
Farm 10^{s}	95	43	45%	691	6100	0
	Σ: 1177	Σ: 422	$\bar{x}$: 36%	$\bar{x}$: 309	$\bar{x}$: 2680	

*: Goat farms. s: Sheep farms. N= herd size. n= sampled animals. %: Percentage of animals analyzed. $\bar{x}$= Mean. Σ= sum.

Table 3. Survey results. Drugs and strategies used by producers for deworming.

Farm ID	Cálculo de la dosis	Animales tratados	Droga utilizada
Farm 1	Individual weight	Entire herd	Ivermectin
Farm 2	Individual weight	Entire herd	ND*
Farm 3	Heaviest animal	Entire herd	Closantel
Farm 4	Individual weight	Entire herd	Closantel
Farm 5	Heaviest animal	Entire herd	Ivermectin
Farm 6	Heaviest animal	Entire herd	ND*
Farm 7	Individual weight	Animals with clinical signs	Levamisole
Farm 8	Heaviest animal	Entire herd	Ivermectina
Farm 9	Individual weight	Animals with clinical signs	Levamisole
Farm 10	Individual weight	Entire herd	Fenbendazole

*ND: Producer did not know or could not recall which drug was used for deworming.

Parasitic load

The distribution of the FEC in each herd shows that the parasitic load of the population tends to cluster around the mean. Isolated points (far from the mean value for each farm) represent the so-called “disseminating individuals,” except in Farm 1. These isolated points (outliers) within each dataset indicate the disseminating individuals in each herd. Additionally, Farms 5 and 8 exhibited low FEC values, likely as an effect of a recent deworming treatment (not reported by the producer). As a result, the entire herd showed a low mean count, with few disseminating individuals observed (Figure 2).

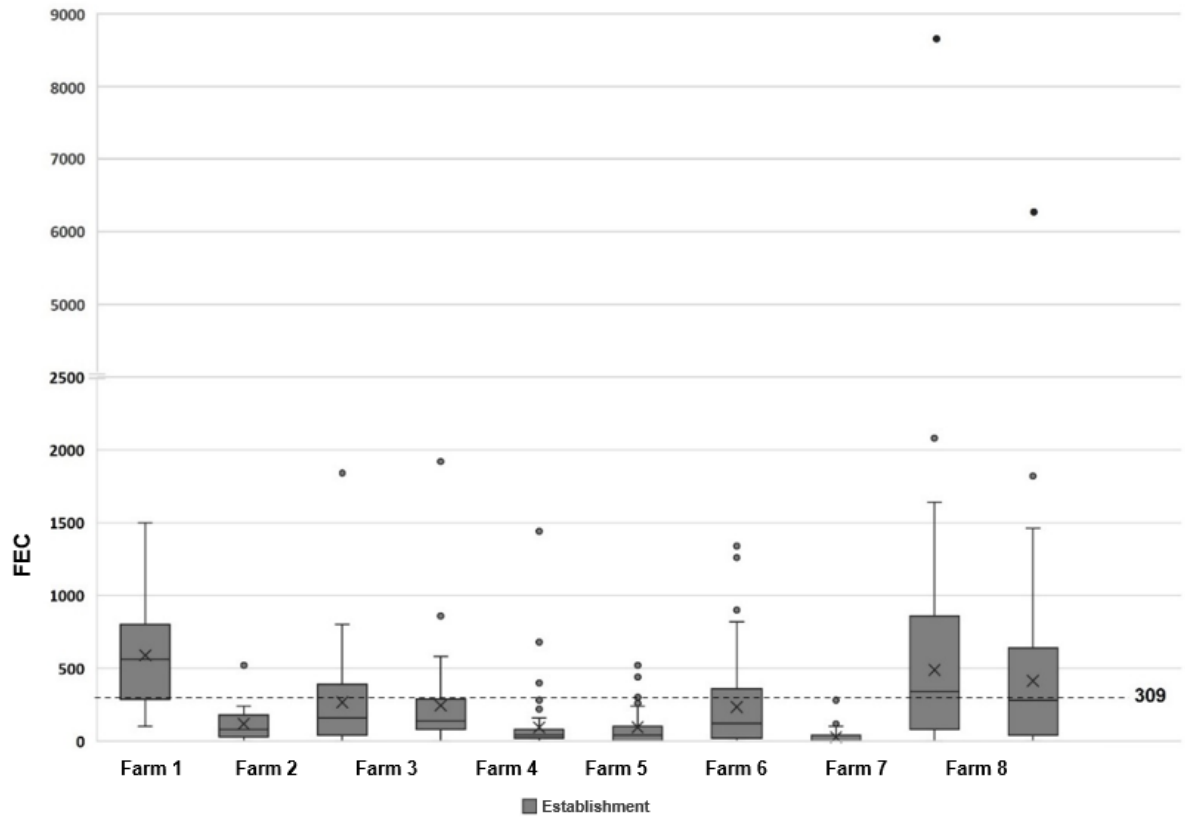

Figure 2. The box-and-whisker plot shows the farms where the parasite load was high (values above the dashed line: 309). The line dividing each box represents the median, while the "x" inside the box indicates the mean value. Additionally, the disseminating individuals in each farm are shown, which appear as "outliers" or isolated points (•) distant from the mean (x).

The distribution of FEC values shows that 71% of the animals had parasite loads below the overall mean (309). The remaining 29% had loads above the mean, with a maximum value of 8600 observed (Figure 3).

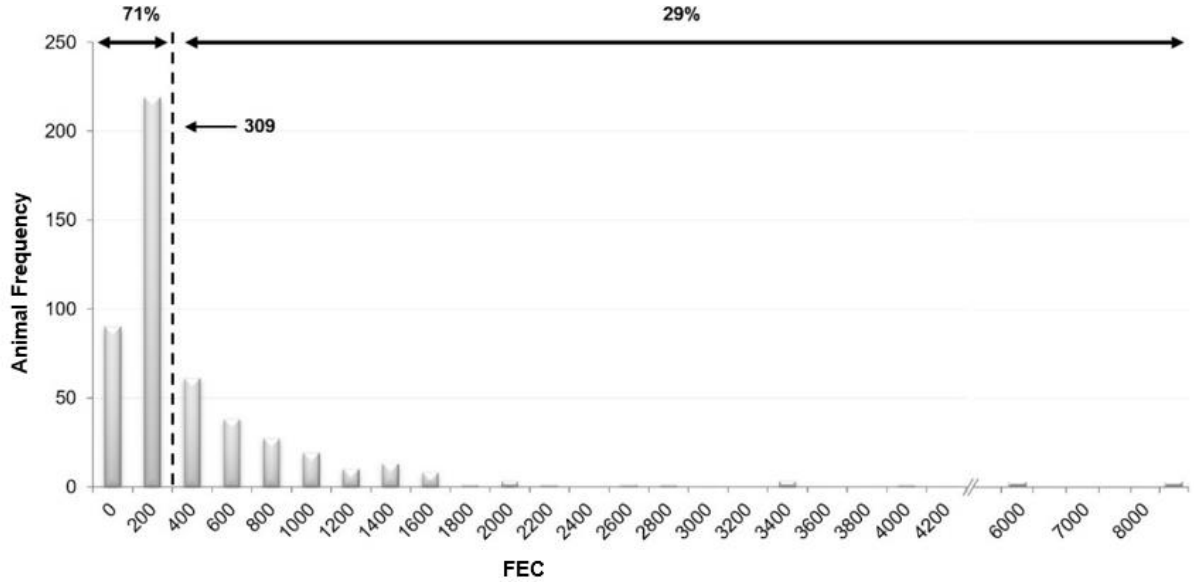

Figure 3. Fecal Egg Count distribution. The dashed line indicates the overall mean FEC value (309). The upper arrows show the 71% and 29% proportions, respectively.

Anthelmintic Resistance Test

Out of all the farms studied, the anthelmintic resistance test could be performed on 20% (2/10) of them (Farms 1 and 7), determined by the availability of a livestock scale at the time of the assays.

In Farm 1, the effectiveness of ivermectin and fenbendazole was 9.7% and 39.3% (corresponding to 90.3% and 60.7% resistance), respectively. In contrast, closantel showed 94.2% effectiveness (5.8% resistance). Data for the group treated with levamisole were unavailable for reasons beyond the scope of this study.

In Farm 7, effectiveness values of 65.0%, 67.8%, and 74.1% (35.0%, 32.2%, and 25.9% resistance) were observed for ivermectin, fenbendazole, and closantel, respectively. In contrast, levamisole showed 96.7% effectiveness (3.3% resistance) (Table 4).

Table 4. Resistance test.

Farm ID		G1-NT	G2-CSL	G3-LVM	G4-IVM	G5-BZM*
Farm 1	N*i*	8	8	-	8	8
	%FECR	-	**94,2**	-	9,7	39,3
	VR	-	0,24	-	0,21	0,18
	CI95 [min-max]	-	79 – 97	-	0 – 67	0 – 76
Farm 7	N*i*	10	10	-	10	10
	%FECR	-	*74,1*	**96,7**	*65,0*	*67,8*
	VR	-	0,30	0,33	0,74	0,35
	CI95 [min-max]	-	0 – 93	88 – 99	0 – 96	0 – 94

G1-NT: Group 1, untreated. G2-CSL: Group treated with Closantel. G3-LVM: Group 3, treated with Levamisole. G4-IVM: Group 4, treated with Ivermectin. G5-BZM: Group 5, treated with Benzimidazole. N*i*: Number of animals. %FECR: Percentage reduction in fecal egg count. VR: Variance of the reduction, representing the variance of the data with respect to the mean and among themselves. CI95: 95% confidence interval. Values in **bold** indicate >94% effectiveness; values in *italics* indicate effectiveness between 65–94%.

Coproculture and Larval Identification

From the four cultured samples, 300 L3 larvae were counted from each (total = 1,200 L3 larvae). Five genera were identified: *Haemonchus* 43% (516) — (*H. contortus* 30% [360] and *H. placei* 13% [156]) — *Cooperia* 35% (420), *Trichostrongylus* 12% (144), *Oesophagostomum* 6% (72), and *Ostertagia* 4% (48) (Figure 4).

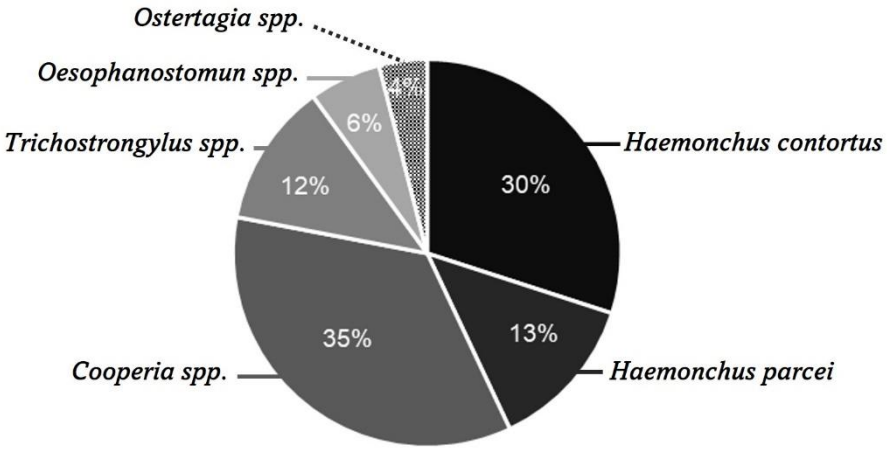

Figure 4. Pie chart showing the proportion of gastrointestinal nematode (GIN) genera and species detected in the coproculture.

Molecular Detection of Genera

The cultured larvae were used to prepare the PCR assay described by Zarlenga et al. (2001). The following species were detected: *Ostertagia ostertagi*, *Haemonchus* spp., *Oesophagostomum radiatum*, *Trichostrongylus colubriformis*, and *Cooperia oncophora*. No nonspecific amplifications were observed in any of the reactions.

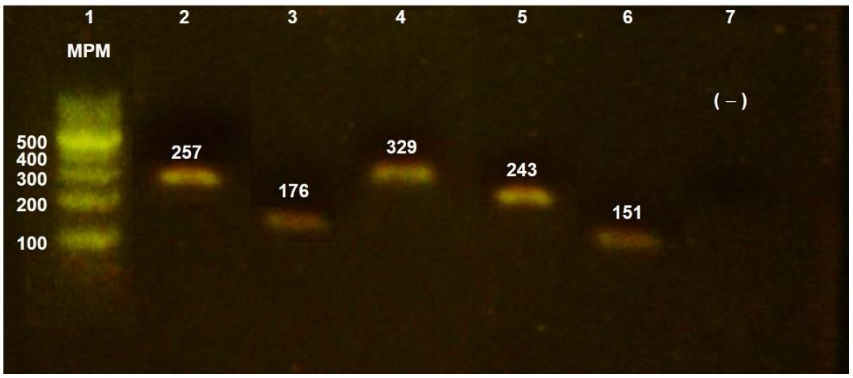

Figure 5. 2% agarose gel showing the bands corresponding to the GIN genera detected.

DISCUSSION

Gastrointestinal nematode (GIN) infections represent a global issue in sheep and goat farming [10]. They have a significant impact in productive regions, and the drive to control them without proper diagnosis has led to the proliferation of anthelmintic drug resistance [10, 35]. This study constitutes the first report describing GINs in sheep and goats from the province of Misiones. It allowed the determination of the average fecal egg count (FEC) in sheep and goats, as well as the implementation of resistance tests and the characterization of the main GIN genera present in the farms studied. In this manner, the data obtained complement the findings previously reported by Fiel et al. (2014) [32], Lobayan et al. (2016) [18], and Da Luz (2020, unpublished data). Our study reveals variability in herd sizes across farms (ranging from 17 to 280 animals), reflecting the smallholder nature [29] of sheep and goat production in the region (Table 2).

The distribution of FEC values varies among animals, with a few individuals responsible for spreading parasites within the farm and the population [6, 36]. Our results show similar variability within and between farms (Figure 2), supporting the idea that treatments should be tailored to each establishment. Thus, determining the herd's FEC makes it possible to identify animals with extreme values (disseminators), which should be the focus of treatment [23]. Furthermore, when analyzing the FEC distribution of all examined animals, most of the population (71%) shows an FEC ≤ 309, while only 29% require antiparasitic treatment, as they are the disseminators of infection within the herd. These findings are consistent with previous reports in sheep [6], [37] and suggest the need for selective or strategic treatment approaches.

The sanitary conditions of sheep and goat farms in Misiones are similar to those described for other provinces in Argentina [28], [38]. These systems are characterized by small-scale, low-efficiency production, feeding mainly on natural pastures, precarious infrastructure, and limited technical assistance [29], [38]. Under these conditions, parasitic infections are treated without laboratory diagnosis, which contributes to the spread of resistance since drug selection is based on tradition, price, or recommendation rather than on efficacy. In this study, two in vivo resistance tests were performed using the Fecal Egg Count Reduction Test (FECRT) [17]. In both farms, populations of parasites resistant to ivermectin were detected (Table 4), a situation already reported in other regions of Argentina [4], [5], [9], [18]–[20], [28], [32], [37]. In addition, one farm showed resistance to fenbendazole, where closantel proved to be the most effective drug. In the second farm, resistance was observed to both closantel and fenbendazole, with levamisole being the effective drug (Table 4). This indicates that each farm presents a distinct situation, and treatments should therefore be based on specific diagnostic testing tailored to each case.

Anthelmintic resistance is a growing global issue driven by the indiscriminate (or improper) use of these drugs, which compromises their long-term efficacy [10]. GIN show high genetic variability, enabling them to quickly adapt to treatments and spread resistance genes within their populations [22]. This phenomenon, documented both worldwide and in Argentina, underscores the need to implement comprehensive health management practices that include laboratory-based diagnoses and selective treatment control strategies. Such measures would help preserve drug efficacy and reduce the spread of resistance within herds [10], [11].

Regarding the genera identified, coproculture analyses revealed the mixed nature of gastrointestinal nematode (GIN) infections, since the different worms inhabit distinct regions of the digestive tract [4] (Figure 4). Among this diversity, the genus *Haemonchus* was predominant (43%), with two species identified: *H. contortus* (30%) and *H. placei* (13%). While *H. contortus* has been widely reported in the literature as the predominant species in sheep and goat herds [4], [11], [17], [18], [23], [26], [28], [32], [38], [39], there are few reports of *H. placei* [17]. Therefore,

ongoing studies are being conducted to further investigate this finding.

In addition, *Haemonchus sp*. and *Cooperia sp*. were detected in 35% of the samples, drawing further attention to the presence of these parasites in the region.

Currently, the use of molecular techniques for monitoring veterinary parasites remains limited in low-resource subsistence farming areas, both in Argentina and worldwide [40]. The evaluation of GIN genera traditionally requires the culture and isolation of L3 larvae [2], although eggs and L1 larvae are sufficient for molecular analyses and may help avoid potential biases associated with culturing [41]. To advance in this area and accelerate the characterization of the most prevalent GINs, a PCR assay was successfully prepared for the detection of *Ostertagia ostertagi*, *Haemonchus sp.*, *Oesophagostomum radiatum*, *Trichostrongylus colubriformis*, and *Cooperia oncophora* [34]. Although additional genera remain to be detected, this assay represents a first step towards modernizing veterinary diagnostics in the region.

CONCLUSIONS

This study highlights the importance of proper diagnosis for the control of GIN infections in the province of Misiones, Argentina. It constitutes the first report describing GINs in sheep and goats from this region, revealing variability in fecal egg count (FEC) among animals and farms, which underscores the need for selective treatments tailored to each establishment. Resistance testing demonstrated the presence of nematode populations resistant to anthelmintic drugs such as ivermectin, fenbendazole, and closantel, confirming the urgency of implementing control strategies based on laboratory analyses to avoid the indiscriminate use of anthelmintics and to prevent the spread of resistant parasites.

Moreover, the characterization of GIN genera—where *Haemonchus* and *Cooperia* were the most prevalent—and the preparation of a PCR assay for the rapid detection of the main parasitic genera represent significant advances for managing these infections.

In conclusion, this study emphasizes the need for more precise health management in the region, incorporating diagnostic-based and strategic treatment approaches to improve animal health and the profitability of local producers. Ultimately, continued monitoring of GIN populations and anthelmintic resistance is essential to adapt control strategies and ensure the sustainability of small ruminant production in the region.

ACKNOWLEDGEMENT

This work was carried out under the Scientific Cooperation Agreement between INTA and UNaM and funded by the INTA projects PE-I044 and RIST-I111 (2023 portfolio).

We thank the Municipality of Profundidad and Mayor Silvita Estigarribia for her support and collaboration.

We are especially grateful to the producers of the Sheep and Goat Production Basin of Southern Misiones, whose willingness to open the gates of their farms made this study possible.

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