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Research Article

Prevalence of Gastrointestinal Strongyle Infestations and *In Vitro* Evaluation of the Efficacy of the Three Anthelmintics on Haemonchus Contortus, Cases of Albendazole, Levamisole and Oxyclozanide in Goats in Supply Markets and in Public Slaughterhouses in Lubumbashi

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Abstract

Gastrointestinal parasitism is a major health problem that affects the productivity of goat farming in the Lubumbashi region. The breeding of small ruminants in this region encounters difficulties related in particular to the management of the herd and the management of diseases such as gastrointestinal verminosis. For a long time, the fight against animal diseases, including verminosis, has been carried out with the use of synthetic chemical molecules. This poorly used chemical control would be at the origin of the development of resistance of certain pathogens. Faced with this constraint, it was planned to study the prevalence of parasitic infestations of GIS, their intensity and to evaluate in vitro the effectiveness of some anthelmintics on the predominant strongyle (*Haemonchus contortus*).

The collection of faecal specimens took place over three months, samples were taken in hasard from 480 goats in slaughter and adult worms (*Haemonchus contortus*) were taken from the abomasum of slaughtered goats.

Qualitative coproscopy was done using Willis' flotation method and quantitative coproscopy using MacMaster's method.

It appears from this study that out of a total of 480 goats from which we collected, 360 were infested, a prevalence of 70%. As for the parasitic intensity, the results of this study reveal that Haemonchus contortus was found in 312 samples out of the 480 or (92.9%) followed by Trichostrongylus colubriformis found in 152 samples out of the 480 or (45.2%) and *Moniezia* spp d years 137 samples on the whole or (40.8%) and at the bottom of the scale *Oesophagostomum columbianum* 43 on the 480 or (12.8%).

The quantification of the different species of parasitic nematodes diagnosed during the present study in terms of OPG (Egg per gram faeces), reveals that *Haemonchus contortus* represents a much larger average of eggs in faeces (3988.6 ± 150.0) while *Oesophagostomum columbianum* has minimum values (483.7 ± 89.9), *Haemonchus contortus* is among the nematos of the most prolific. However, in the majority of cases, it was a multi-parasitism.

The efficacy of (albendazole, levamisole and oxyclozanide) was tested *in vitro* at concentrations 2.0; 1.0; 0.5; 0.25; 0.125 mg/mL. At the first three concentrations, all extracts showed significant differences from negative controls (DMSO and PBS). As a result, levamisole was more effective at concentrations of 2 milligrams and 1 milligram per milliliter (90 and 84%) with statistically significant differences compared to the other two drugs. For the three drugs tested, larval paralysis was observed only with the first three concentrations, i.e., 2, 1 and 0.5 milligrams per milliliter.

Keywords: Prevalence; Gastrointestinal Strongyle; Goat; Anthelmintic; Lubumbashi

Abbreviation

ANOVA : Analysis of Variance; LD50: Lethal dose; DMSO: Diméthylsulfoxyde; GIP: Gastrointestinal parasites; IC50: Median Inhibitory Concentration; Kg: Kilogram; KH_2PO_4: Potassium Hydrogen Phasphate; mg: Milligram; ml: millilitre; OPG1: Number of Eggs Per Gram; PBS: Phosphate-Buffered Saline; PCR: Polymerase Chain Reaction; SD: Deviation Stantard; SGI: Gastrointestinal Strongyles

Introduction

Goat rearing is an important activity which enables farmers to provide additional income, the only way for many families to contribute to the realization of projects or to meet certain expenses of daily life. However, this breeding encounters difficulties, particularly related to the management of the herd and the management of diseases such as gastrointestinal verminosis. Infestations by parasitic nematodes of the digestive tract represent one of the dominant pathologies of animals raised in Lubumbashi and its surroundings [7,19].

For a long time, the fight against animal diseases including verminosis has been carried out with the use of synthetic chemical molecules. This poorly used chemical control is thought to be at the origin of the development of resistance in certain pathogens. Faced with this constraint, it is necessary to evaluate the effectiveness of anthelmintics in common use [2,13,14]. Most of the anthelmintics available on the local market are limited to benzimidazoles and macrocyclic lactones. Unfortunately, their repeated use in the long term allows parasites to adapt by developing irreversible resistance to the majority of families of the moles [1].

The objective of this study was to study the prevalence of GIS, their intensity and to contribute to the documentation related to the efficacy of anthelmintic drugs marketed and used in livestock farms in Lubumbashi.

Specifically, we will

- Study the prevalence of parasitic infestations in goats in killings and markets selling live animals;
- Determine the intensity of the infestation taking into account the different parasitic nematodes diagnosed;
- Determine the degree of infestation of each parasitic nematode;
- To evaluate in *vitro* the efficacy of anthelmintic drugs available in veterinary pharmacies in Lubumbashi.

Middle

This study was carried out in Lubumbashi, capital of Haut-Katanga province, itself located in southeastern DR Congo. The city of Lubumbashi is subdivided into 7 communes spread over an area of 747 km 2, with an estimated population of about 2,584,000 inhabitants or a density of 3459 inhabitants/km^2 in 2021, according to statistical data published by the World Bank [10].

It is located between 11°20' and 12°00' South latitude and 27°10' and 27°34' East longitude and is situated at an average altitude of 1224 m. The average annual rainfall is 1243 mm, the average annual temperature is 41°C.

The collection of faeces samples for coprological analysis was carried out in 12 killings in Kamalondo commune and 6 live goat outlets (M'zee market, Mimbulu/Matshipisha market, Camp Vangu market, Rail market, Kamalondo market and Zambia market), whose locations are shown in figure 1.

Due to the regularity of the slaughter carried out and the large number of animals slaughtered, the killing of the Mzee market served as a place for us to collect worms in order to carry out the analysis of the effectiveness of anthelmintic drugs available in pharmacies and veterinary drug sales outlets in the city of Lubumbashi.

The coprological examinations were carried out in the laboratory of the Veterinary University Clinics of the Faculty of Veterinary Medicine at the University of Lubumbashi. The pharmacological studies were carried out at the Laboratory of Pharmacology, Therapeutics and Toxicology of the same Faculty and University mentioned above.

Material

- Harvesting equipment for faeces and nematodes: gloves, sterile collection bottles, a cooler and a plastic bin;
- Coprological analysis equipment: an optical microscope (Leitz Wetzler), distilled water, saline solution (40%), microscopic slides and slides, a McTMaster cell, laboratory glassware, Pasteur pipettes, sieves of different meshes (1 mm, 500, 212, 75 and 38 µm), wooden spatulas and a precision balance;
- Chemicals: anthelmindrugs (albendazole, levamisole and oxyclozanide), saline buffer solution or PBS (108.3 g KH 2 PO 4; 35.6 g K_2HPO_4; 1L of deminated water), saline.

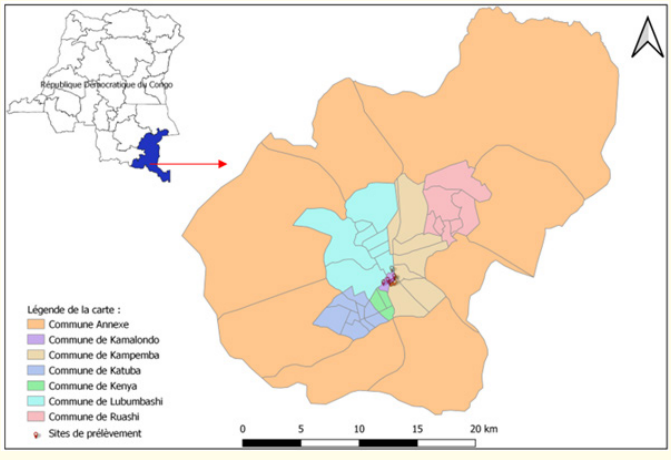

Figure 1: Presentation and location of the city of Lubumbashi.

Methods

Parasitism in goats in killings

Collection of faecal specimens

The collection of faecal specimens took place during a period of three months (October to December). Faecal samples were taken at random from live goats and animals awaiting slaughter in the killings. The samples were taken directly from the rectum of each animal and placed in sterile and labelled sample bags, then stored in a cooler and finally sent to the laboratory for parasitological analysis. When the analysis was not carried out immediately, the rottines were stored in the refrigerator at 4°C pending examination.

Coprological examinations

The search forhemint eggs and their count were based respectively on qualitative and quantitative coprology carried out under the microscope (coproscopic methods). Coproscopy is the most widely used basic method for parasitological diagnosis. It is a question of observing in the faeces the eggs of strongyles laid by adult worms present in the digestive tract of the animal.

Coproscopie qualitative

For qualitative coprological analyses, we used the Willis flotation method. The latter is a method of enrichment that consists of concentrating worm eggs or larvae in feces, so that, even in small numbers, they can be detected [20].

The technique of this method can thus be summarized as follows.

- Prepare a saturated solution of sodium chloride 40%.
- Weigh 4 g of the stool of the sample to be analyzed.
- Crush the stool taken from a porcelain mortar using a pestle, add 15 ml of 40% sodium chloride solution.
- The faecal slurry obtained was filtered over a beaker and the residue retained on the sieve was washed by adding the dense solution, so that the total volume of the suspension is about 60 ml;
- The solution thus obtained was thoroughly filled into a test tube. Above the tube, we placed a lamella, taking care not to include air bubbles under it. The tube was thus allowed to rest for 15 to 20 minutes, after which the slide was removed at once and placed on an object slide and examined under an optical microscope (Leitz, eyepiece 10X, objectives 10 and 40).

Coproscopie quantitative

Coproscopy only makes sense if it is quantitative. The amount of eggs is generally proportional to the number of worms present, except in animals with a very high level of infestation. This count therefore indirectly reflects the importance of parasitism. For the quantification of eggs per gram of fecal matter (OPG), we used the McMaster technique [20]. It consists of crushing 4 g of fecal matter taken directly from the rectum, adding 56 ml of a saturated solution of sodium chloride (which allows the flotation of eggs contained in the feces), filter and fill the 2 cells of the McMaster blade. Each cell has a known volume of 0.15ml so, as the faeces are diluted to 1/15, the number of eggs counted is that contained in one hundredth of a gram of faeces. To obtain the number of eggs per gram, the result obtained when counting on one compartment is multiplied by 100 and for both compartments by 50.

OPG = Number of eggs in both compartments x 50.

Effect of drugs on motility of adult worms

The adult worms (*Haemonchus contortus*) were taken from the abomasum of goats slaughtered at the M'zée market massacre (City of Lubumbashi). Immediately after slaughter, the abomasum was collected and transported to the laboratory. The parasites were then collected, washed and stored in phosphate-buffered saline (PBS). The experiment was conducted using the technique used by Eguale., *et al.* [6]. Ten live worms were placed in petri dishes containing 2.0; 1.0; 0.5; 0.25; 0.125 mg/mL of albendazol, levamisol and oxyclozanide dissolved in DMSO (dimethyl sulfoxide) and then diluted in PBS and PBS alone as a negative control, in a total volume of 4 ml. Three repeats per concentration of each treatment were used. After 24 hours, the parasites were cleaned and then hung in the PBS for 30 minutes for possible recovery of the parasite's motility. The number of mobile (alive) and immobile (dead) worms was counted using a magnifying glass. The results of each concentration were recorded. The death of the worms was noted by the absence of motility during an observation period of 5 to 6 seconds. A mortality index was calculated by dividing the number of dead worms by the total number of worms per petri dish.

Statistical analysis

Prevalence was estimated as a percentage of the number of infected animals relative to the total number of animals examined. Data from the motility test on adult worms (*H. contortus*) after 24 hours of incubation and statistical significance analysis were performed using the ANOVA (Analysis of Variance) test with GraphPad Prism 6.0 (Graphpad Software, Inc., San Diego, CA, USA) for a probability ($p > 0.05$).

Results

Parasitism of goats in killings and outlets of live animals

Tables 1, 2 and 3 below present the results for infestation of animals prior to slaughter. We have synthesized in these tables data on the prevalence of infestations in the animals examined, the intensity of the different parasites diagnosed and their quantification (OPG).

Goat killings and markets	Animals examined	Infested animals	Frequency (%)
Kamalondo 1	22	18	81,8
Kamalondo 2	24	16	66,7
Kamalondo 3	20	15	75,0
Kamalondo 4	26	14	53,8
Kamalondo 5	10	10	100,0
Kamalondo 6	21	10	47,6
Kamalondo 7	19	14	73,7
Kamalondo 8	17	12	70,6
Kamalondo 9	17	10	58,8
Kamalondo 10	19	9	47,4
M'zee 2	109	89	81,7
Mimbulu	24	17	70,8
Rail	37	21	56,8
Kamalondo	52	29	55,8
Camp Vangu	36	32	88,9
Zambia	27	20	74,1
Total	480	336	70,0

Table 1: Prevalence of goat infestations in animal killings and outlets.

In this study, the different killings and points of sale of goats were identified, the number of animals in each entity was taken followed by the determination of the frequency of animals infested with gastrointestinal nematodes in the commune of Kamalondo (Table 1). These results show that out of a total of 480 goats, 360 were infested, i.e., 70%. The highest prevalence (100%) was found in Kamalondo 5 while the minimum prevalence was found in Kamalondo 10 (47.4%). The results obtained are therefore necessary to determine the intensity of the different species of nematodes in infested animals (Table 2).

Parasitic nematodes	Number of animals infested	Frequency (%)
Haemonchus contortus	312	92,9
Trichostrongylus colubriformis	152	45,2
Moniezia spp	137	40,8
Strongyloides papillosus	96	28,6
Teladorsagia circumcincta	92	27,4
Nematodirus spp	57	17,0
Oesophagostomum columbianum	43	12,8

Table 2: Intensity of different nematode species in infested animals (n = 336).

These results show the intensity of different species of nematodes in infested animals. This table summarizes in descending order, the most predominant parasitic nematodes. *Haemonchus contortus* occupies the first place with 92.9% representativeness while *Oesophagostomum columbianum* represents a low intensity compared to all parasites identified in goats.

Parasitic nematodes	Average ± SD
Haemonchus contortus	3988,6 ± 150,0
Trichostrongylus colubriformis	1121,7 ± 99,7
Moniezia spp	784,8 ± 90,9
Strongyloides papillosus	778,5 ± 94,7
Teladorsagia circumcincta	563,4 ± 97,8
Nematodirus spp	537,2 ± 88,5
Oesophagostomum columbianum	483,7 ± 89,9

Table 3: Eggs per gram of faeces (OPG) of diagnosed nematodes.

This table gives the quantification of the different species of parasitic nematodes diagnosed during this study. The worms are quantified in terms of OPG (egg per gram feces). It shows that *Haemonchus contortus* represents a much larger average of eggs in faeces while *Oesophagostomum columbianum* has minimum values.

Effects of albendazole, levamisole and oxyclozanide

The results of the evaluation of the efficacy of anthelmintic drugs are presented in Table 4 below. Figure 2 illustrates the IC50 values of the concentrations tested.

Values are expressed on average ± SD; negative controls DMSO and PBS all showed a mortality percentage of 0%. The same su-

Concentration (mg/mL)	Number of worms exposed to drugs	Average ± SD of moving worms (% mortality)		
		Albendazole	Lévamisole	Oxyclozanide
2	10	3,3 ± 0,6 (67)[a]	1,0 ± 0,0 (90)[b]	3,3 ± 0,6 (67)[a]
1	10	5,6 ± 1,2 (44)[a]	1,6 ± 0.6 (84)[b]	6,3 ± 1,2 (37)[a]
0,5	10	6,3 ± 1,2 (37)[a]	6,3 ± 1,2 (37)[a]	6,3 ± 1,2 (37)[a]
0,25	10	10,0 ± 0,0 (0)[a]	10,0 ± 0,0 (0)[a]	10,0 ± 0,0 (0)[a]
0,125	10	10,0 ± 0,0 (0)[a]	10,0 ± 0,0 (0)[a]	10,0 ± 0,0 (0)[a]

Table 4: Effects of drugs on *Haemonchus contortus.*

perscript letters show that there is no statistical difference in the results obtained while the different letters show a statistically significant difference between the results (ANOVA; $p < 0.05$).

Three commercial anthelmintic drugs (albendazole, levamisole and oxyclozanide) were tested at different concentrations. At the first three concentrations, all extracts showed significant differences from negative controls (DMSO and PBS). As a result, levamisole was more effective at concentrations of 2 milligrams and 1 milligram per milliliter (90 and 84%) with statistically significant differences compared to the other two drugs. The effectiveness of albendazole is close to that of oxyclozanide. For the three drugs tested, larval paralysis was observed only with the first three concentrations, i.e., 2, 1 and 0.5 milligrams per milliliter. From 0.25 milligrams, the three products tested showed no effect on *Haemonchus contortus.* The efficacy results of the drugs tested are shown in figure 2 below.

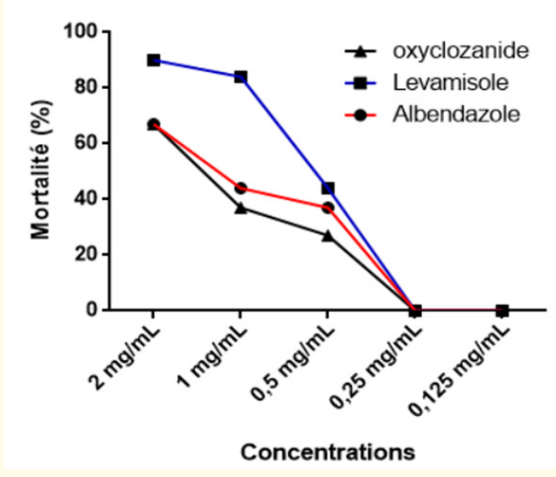

Figure 2: Effect of anthelmintic drugs on *H. contortus.*

Discussion

The general objective of this study was to contribute to the literature related to the efficacy of anthelmintic drugs marketed in Lubumbashi. The prevalence of infested animals in goat slaughterhouses and outlets, the intensity of infestations following parasitic nematodes, the quantification of worms and the study of the efficacy of three anthelmintic drugs were determined.

These results show that of the 480 goats examined, 336 were infested, representing a prevalence of 70% (Table 1). Gastrointestinal parasitism is a major health problem that affects the productivity of goat farming worldwide [17,22,23]. This can be attributed to the exposure of goats to areas contaminated with different types of parasites.

However, the overall prevalence of IPM of 70% observed in this study is similar to that of IPM in goats in Cameroon (90.04%) [18], Bangladeshsh (77.0%) [11] and India (5-1.89%) [4]. The rainy season as well as the mode of exploitation of goats can be blamed. In this study, samples were taken and analyzed during the rainy season.

As for the intensity of diagnosed parasites, the results of this work reveal that *Haemonchus contortus* (92.9%) is the most common parasite in goats, followed by *Trichostrongylus colubriformis* (45.2%) and *Moniezia* spp. (40.8%) among those observed in the present study (Table 2). Our observations are similar to those of some other studies conducted in Africa and other continents, describing *Haemonchus contortus* as a major problem in small ruminants [5,8,15]. However, several authors have already reported that *Eimeria* spp. is the most common parasite among gastrointestinal parasites in goats [12,21,25,26].

The results of this work reveal that all animals sold, or even those ready for slaughter, are infested with several nematodes. *Haemonchus contortus* alone is more dominant with an average OPG of 3988.6 ± 150.0 (Table 3). This parasite is among the most

prolific nematodes. In the majority of cases, it is a multi-parasitism. Our results are similar to those obtained by Hassan., *et al*. (2019) [9]. This can be attributed to the exposure of goats to pastures contaminated with different types of pests.

In connection with the evaluation of the anthelmintic activity of different drugs (Table 4), this study showed that albendazole, levamisole and oxyclozanide are effective *in vitro* against *Haemonchus contortus* up to the concentration of 0.5 mg/mL with statistically significant differences compared to dimethylsulfoxide and PBS. This fact recalls that the minimum concentration inhibiting the motility of the nematode for the three products is 0.5 mg/mL. It should be noted that the effect of levamisole is significantly higher compared to the other two drugs tested. The different effects observed could be explained by the mechanism of action of these products.

Indeed, albendazole (Benzimidazoles) acts by binding with high affinity to beta-tubulin and inhibits the polymerization of microtubules, thus modifying the tubulin-microtubule balance. Beta-tubulin is a component protein of microtubules, which are important structures of the cytoskeleton and mitotic spindle in eukaryotic cells [3]. Levamisole is a cholinergic agonist that acts on nicotinic acetylcholine receptors (NAChR), thereby disrupting neuromuscular transmission resulting in worm paralysis due to sustained muscle contraction [16]. Oxyclozanide is a proton ionophore that acts as a specific decoupler of mitochondrial oxidative phosphorylation thus disrupting the metabolism of the parasite [24].

Conclusion

In conclusion, our study was carried out with the aim of determining the prevalence of SGI and evaluating the effectiveness of anthelmintic drugs available on the market in Lubumbashi, vis-à-vis *Haemonchus contortus* (parasitic nematode of small ruminants) which predominates in goat farming in Libumbashi.

At the end of these investigations, it was observed that SGI predominate goat farms in Lubumbashi and that *Haemonchus contortus* has a high intensity. We observed that the molecules used still show some effectiveness in general. However, there is a clear difference in efficacy, with levamisole appearing to be more effective than albendazole and oxyclozanide.

Furthermore, it should be noted that if these anthelmintics continue to be abused, in particular by poor packaging and poor storage of veterinary products in pharmacies and commercial homes, the practices of farmers such as non-compliance with doses (or even dosages), repeated use of a single anthelmintic drug throughout the time, otherwise the non-alteration of molecules could lead to the installation of resistance to these molecules. With all the consequences and economic losses that will be recorded by small farmers, the decrease in the zootechnical performance of animals and a decrease in productivity.

Faced with all these eventualities, we can recommend that goat breeders and traders think about rigorous veterinary monitoring, particularly in the reasonable use of anthelmintic drugs in animals on farms or even in places where live animals are sold to ensure a good yield at slaughter.

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Conflict of Interest

None.

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