



Strongyloides sp resistant to albendazole and levamisole in buffaloes from Mexico

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ABSTRACT

Objective. The anthelmintic efficacy of albendazole and levamisole in *Strongyloides* sp. populations was assessed in buffalo calves (*Bubalus bubalis*) with natural infections in the Mexican tropic. **Materials and methods.** 45 buffalo calves were included in the study and distributed into three experimental groups (15 specimens each), according to the excretion of eggs of *Strongyloides* sp. per gram of faeces (EPG), namely: (a) Control group: without deworming; (b) BZ group (benzimidazoles), which received oral albendazole (10 mg/kg); and (c) IMIDA group (imidazothiazoles) that received levamisole (8 mg/kg) subcutaneously. Faeces were obtained directly from the rectum on days zero (pre-treatment) and 10 (post-treatment) to determine the EPG of each animal. The samples were processed using the McMaster technique. The EPG results were analysed using the RESO software to determine the percentages of egg reduction and their 95% confidence intervals (95% CI). **Results.** The BZ group exhibited 94% reduction in EPG (95% CI = 87-97), and the IMIDA group exhibited 95% reduction in EPG (95% CI = 84-99). **Conclusions.** This is the first report in Mexico on the occurrence of *Strongyloides* sp. populations resistant to benzimidazoles and imidazothiazoles in naturally infected buffaloes from the Mexican tropic.

Keywords: Drug resistance; occurrence; *Strongyloidosis*; tropical zones (Source: USDA).

RESUMEN

Objetivo. Se evaluó la eficacia antihelmíntica de albendazol y levamisol sobre poblaciones de *Strongyloides* sp. en bucerros (*Bubalus bubalis*) con infecciones naturales en el trópico Mexicano. **Materiales y métodos.** Se incluyeron 45 bucerros, que fueron distribuidos en tres grupos experimentales de acuerdo a la excreción de huevos por gramo de heces (HPG) de *Strongyloides* sp. (n=15): a) Grupo control: sin desparasitación, b) Grupo BZ (benzimidazoles) que recibió albendazol a razón de 10 mg/kg vía oral, y c) Grupo IMIDA (imidaziotazoles) dosificación de levamisol a

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razón de 8 mg/kg vía subcutánea. Para determinar los HPG de cada animal, se obtuvieron heces directamente del recto los días 0 (Pre-T) y 10 (PT). Las muestras fueron procesadas mediante la técnica de McMaster y los resultados de HPG se analizaron mediante el programa RESO para conocer el porcentaje de reducción de huevos y sus intervalos de confianza al 95% (IC95%). **Resultados.** El grupo BZ registró un 94% de reducción de HPG (IC95%= 87–97) y el grupo IMIDA registró 95% de reducción en el HPG (IC95%= 84–99). **Conclusiones.** Se reporta por primera vez en México la presencia de poblaciones de *Strongyloides* sp. resistentes a benzimidazoles e imidazotiazoles en bucerros del trópico mexicano infectados naturalmente.

Palabras clave: Resistencia; prevalencia; *Strongyloidosis*; zonas tropicales (Fuente: USDA).

INTRODUCTION

Strongyloidiasis is a parasitic disease caused by a group of nematode parasites belonging to the genus *Strongyloides*, exhibiting a worldwide distribution. These parasites infect a wide range of hosts, and the species *Strongyloides papillosus* has been reported in ruminants (1). The species *S. stercoralis* is probably the most important, given that it affects humans and companion species, causing an intestinal anthroponotic disease, common in tropical and subtropical regions (2).

In cattle, *S. papillosus* is characterised by completing its life cycle by free-living generations or alternate parasites, and by establishing itself in the small intestine, producing pruritus, localised oedema, diarrhoea, loss of appetite, weight loss, retarded growth in young animals, and even sudden deaths (3). Worldwide and in Mexico, the occurrence of *Strongyloides* sp. has been reported as a common parasite in ruminants (sheep, goats, and cattle), but of little importance, since its occurrence is sporadic and with little or low elimination of eggs in the faeces. However, it is a common parasite in buffaloes, causing serious infections in animals aged less than one year (4).

The control of gastrointestinal nematodes in ruminants has been based mainly on the administration of anthelmintic drugs, which has led to the appearance of parasites resistant to the main families commercially available (5,6). However, cases of resistance in populations of *Strongyloides* sp. affecting ruminants, specifically buffalo species, have been scarcely reported. The present study assessed the anthelmintic efficacy of albendazole and levamisole in populations of *Strongyloides* sp. in buffalo calves (*Bubalus bubalis*) naturally infected in the Mexican tropic.

MATERIALS AND METHODS

Ethical aspects. The present study was approved by the Ethics Committee of Juárez Autonomous University of Tabasco (File: 0628). The handling and collection of samples were performed by veterinarians, in compliance with the ethics code of the Federation of zootechnical veterinarian doctors of Mexico. (2020).

Location. This research was conducted in a dual-purpose buffalo production farm, located in the state of Tabasco, Mexico. The climate of the area is warm humid with rains in summer, an average annual temperature of 27°C, and rainfall ranging from 1500 to 3000 mm per year (7).

Experimental procedure. The protocol of the field technique advocated by the World Association for the Advancement of Veterinary Parasitology (WAAVP) was used to assess faecal egg count reduction (8). The technique is based on the detection of the reduction in the number of eggs eliminated in animal faeces after providing anthelmintic treatments, which should be performed according to the recommended doses.

Study animals. Seventy-five female and male Murrah buffalo calves and their crosses (Mediterranean and Jafarababi), aged one to four months, with average weight of 45±2 kg and without deworming were randomly included, at least four weeks before the start of the study.

Faeces samples were collected on day zero (pre-treatment [Pre-T]) to determine the occurrence of *Strongyloides* sp. and the amount of EPG, as well as other gastrointestinal parasites. Based on the amount of EPG, 45 calves, with at least 150 EPG of *Strongyloides* sp., were selected for the study.

Experimental design. The selected animals (n=45) were distributed into three homogeneous experimental groups based on their elimination of EPG. The groups had 15 specimens each, they were:

(a) Control group: without deworming ; (b) BZ group (benzimidazoles), which received 10 mg/kg of oral albendazole (Bovex® Company, California); and (c) IMIDA group (imidazothiazoles) that received 8 mg/kg of levamisole (Fosfamisol®, Biogénesis Bagó) subcutaneously. The individual doses were calculated based on the live weight of the animals, and the corresponding treatments were provided. On day 10 (post-treatment [PT]), faeces samples were collected from the experimental animals to obtain the amount of EPG after the administration of anthelmintics per group.

Samples and processing. Faeces samples were obtained directly from the rectum of the animals using plastic bags. The samples were identified and stored in refrigerator conditions. The faeces were processed using the modified McMaster technique. Faecal cultures were performed per experimental group on day zero (Pre-T) and day 10 (PT). These cultures were incubated at room temperature in the laboratory in order to obtain infective larvae for determining the nematode genera.

Statistic analysis. The data relating to EPG from the Pre-T day were analysed using descriptive statistics to form the experimental groups and characterise the occurrence of *Strongyloides* sp. The elimination average and intensity ranges by type of parasite were calculated. Additionally,

EPG of each parasite was classified into three categories, namely: negative (0 EPG); low to intermediate (50 to 950 EPG); and high (>1000 EPG). Subsequently, the prevalence by type of parasite was determined.

The reduction percentages and confidence intervals (95% CI) by PT experimental group were obtained using the RESO.exe® programme. The presence of anthelmintic resistance was determined when two criteria were met, i.e., (a) reduction in EPG less than 95%, and (b) 95% CI value less than 90%.^[8]

RESULTS

The population of *Strongyloides* sp. was resistant to the families assessed, although a reduction of 94% was observed for the family of BZ (albendazole), and 95% for the family of IMIDA (levamisole). In both cases, the lowest 95% CI values were less than 90%. Therefore, the resistance to the two families of anthelmintics was determined (Table 1).

The larvae obtained from the faecal cultures confirmed the presence of the genus *Strongyloides*; however, few larvae were obtained in all cultures, so the data are not presented. The amount of EPG in the pre-treatment indicated that, of the 75 buffaloes sampled, the majority were positive (prevalence) for the genus *Strongyloides* (86.6%), with an intensity ranging from 50 to 11900 EPG (Table 2). Additionally, nematodes of the Strongylidae family, *Toxocara* spp. and *Eimeria* spp. were found in buffalo calves, with a lower proportion and intensity of infection.

Table 1. Diagnosis of resistance of *Strongyloides* sp. at a buffalo production farm in a tropical Mexican area.

Group	Treatment	Average EPG (range)		EPG reduction (%)	Confidence interval (95%)	Diagnosis
		Pre-treatment	Post-treatment			
Control (n = 15)	-----	1630 (500-6000)	2889 (300-6950)	-----	-----	-----
BZ (n = 15)	Albendazole	1627 (750-4233)	177 (0-450)	94	87-97	Resistant
IMIDA (n = 15)	Levamisole	1703 (400-8100)	136 (0-950)	95	84-99	Resistant

Table 2. Distribution and elimination of eggs of gastrointestinal parasites per gram of faeces in buffaloes with natural infections.

Parasite	Eggs per gram of faeces (n = 75)		Category number of buffalo calves (EPG range)			
	Average	Range	Prevalence of positive specimens (%)	Negative	Low and Intermediate	High
<i>Strongyloides</i> sp.	1201	0 - 11900	86.6 (65/75)	10	34 (50 - 950)	31 (1000 - 11900)
Family Strongylidae	40	0 - 450	29.3 (22/75)	53	22 (50 - 450)	-----
<i>Eimeria</i> spp. (OPG)*	1292	0 - 20500	40.0 (30/75)	45	15 (50 - 700)	15 (1100 - 20500)
<i>Toxocara</i> spp.	813	0 -14550	49.3 (37/75)	38	23 (50 - 850)	14 (1350 -14550)

Note. *Oocysts per gram of faeces.

DISCUSSION

The detection of anthelmintic resistance has been mainly focused on parasites that affect sheep, goats, and cattle (9). Most of those studies have been addressed to the family Strongylidae, with the parasite *Haemonchus contortus* being the most studied (10) and used as a study model for assessing anthelmintic resistance *in vitro* and *in vivo* (11). Anthelmintic resistance occurs as a consequence of inappropriate and indiscriminate use of anthelmintics. One of the main factors associated with this phenomenon has been the frequent use and incorrect dosage of the products (under- and overdosing)(12,13).

The specific genetic mechanisms of resistance to each deworming group have been widely studied and documented (11). However, it is necessary to perform resistance diagnoses for each family or species of parasites, since the inherent characteristics of parasites such as biotic potential, life cycle, type of reproduction, and habitat differ among them. Therefore, resistance varies between each family or species (10).

The occurrence of *Strongyloides* sp. multi-resistant to two families of anthelmintics was determined in the present study. This is the first report that assessed the resistance of these nematodes in buffaloes from Mexico. *Strongyloides* sp. has been reported as a common parasite in natural infections of buffaloes (4,14). However, as in other ruminants, anthelmintics are generally used to control gastrointestinal nematodes of the Strongylidae family. As a consequence, *Strongyloides* sp. is subject to untargeted control (15).

In the present study, the efficacy of levamisole and albendazole for the control of *Strongyloides* sp. in buffaloes from Mexico was $\geq 94\%$, which indicates that resistance was in its initial stage. Few studies have reported resistance in parasites that affect buffaloes (16,17). Therefore, the real situation regarding the existence of anthelmintic resistance to the main anthelmintic families in buffalo populations is not known.

In sheep, frequent use and under- and overdosing of anthelmintic drugs have been determining factors in the emergence of resistance (18). In the buffalo production farm assessed in the

present study, both products had been used for six years, being administered every 14 days in the initial stages of the calves' lives, which presupposes a high selection pressure in the parasites and decreased refuge use.

Likewise, *Strongyloides* sp. has a high biotic potential when transmitted to buffaloes orally or percutaneously (19). This biotic characteristic during transmission could favour the parasite to remain in a refuge state and promote less selection of resistant nematodes (20). However, if the frequent use of these families of anthelmintics continues in buffalo production farms, it could cause a decrease in efficacy, thus limiting the use of these families for the control of this species of nematode.

On the other hand, little is known about the epidemiology of *Strongyloides* sp. in the Mexican tropic (4), although its occurrence in the species is related to age groups worldwide. In the present study, 86.6% of the young buffaloes assessed tested positive for *Strongyloides* sp., with excretions ranging from 50 to 11900 EPG; however, none of the buffalo calves exhibited clinical signs of parasitism. It is recommended that further studies assess the damage caused by this species of nematodes in growth and productivity of buffalo calves, as well as the economic impact in the Mexican tropic.

One of the main measures that can delay the failure of anthelmintic drugs consists of identifying high-risk practices in each livestock farm. Hence the importance of appropriate diagnoses and selection of suitable anthelmintics, especially for buffaloes, since the information about this process is scarce. The present study contributes to the knowledge about the topic assessed, and reports the presence of anthelmintic resistance in common parasites affecting the buffalo species.

Due to the high frequency and intensity of *Strongyloides* sp. in buffalo calves, the presence of other parasites (family Strongylidae, *Toxocara* spp. and *Eimeria* spp.), and the identification of anthelmintic resistance in *Strongyloides* sp., it is imperative to establish control programmes for parasites in buffaloes of the Mexican tropic. In conclusion, the present study reports the occurrence of the intestinal nematode *Strongyloides* sp. resistant to two families of anthelmintics in buffalo calves with natural infections raised in southern Mexico.

Conflicts of interest

The authors declare that there is no conflict of interest related to this publication.

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