Control and prevention of nematodiasis in small ruminants: background, challenges and outlook in Mexico

David Emanuel Reyes-Guerrero a
Agustín Olmedo-Juárez a
Pedro Mendoza-de Gives a*


*Corresponding author: pedromdgives@yahoo.com; mendoza.pedro@inifap.gob.mx

Abstract:

Nematode parasites are an ongoing challenge in livestock production. Pharmaceutical anthelmintics are effective but pose their own risks. This is an overview of nematodiasis in small ruminants in Mexico focusing on the main problems faced by producers to maintain productivity. It includes general information on gastrointestinal nematodes and their effects on animal health and productivity. It also summarizes the main challenges faced by livestock producers in combating these parasites and current control and prevention strategies, including pharmaceuticals, anthelmintic resistance, grazing management, selective deworming, protein nutritional strategy, vaccination, and selection of animals genetically resistant to nematodes. The potential use of plants and compounds with nematocidal activity, and nematophagous fungi as biological control agents are also covered. Research by the Helminthology Department of the CENID-SAI of the INIFAP is highlighted, and a comprehensive nematode control method is proposed that targets different control strategies at specific nematode developmental stages. Controlling nematodiasis in small ruminants is vital to the success of production systems since it negatively affects animal health and producer results. Continued development of new nematode control options holds promise for successful long-term management of this disease.

Key words: Nematodiasis, Parasites, Sheep, Goats, Control, Prevention.
Introduction

Importance of sheep and goat production in Mexico

Small ruminant production in Mexico represents a significant source of animal protein in human diets\(^1\), and generates approximately 50,000 direct and indirect jobs that benefit as many as 400,000 families\(^2\). However, animal health and producer income are adversely affected by poor quality pastures\(^3\), high feed costs\(^4\), extreme weather driven by climate change\(^5\), and a suite of nematode parasites.

Gastrointestinal nematodes in small ruminants

Gastrointestinal nematodes (GIN) are cylindrical worms that inhabit the digestive tract of ruminants. There are considered significant parasites in the livestock industry, mainly in extensive systems, in both tropical and temperate climates\(^6\). Adult parasites copulate and produce immense quantities of eggs which are released into the environment in the feces. Here they develop into infective larvae (L3) that contaminate pastures. Infection occurs when animals consume grass contaminated with larvae\(^7\). The principal GINs in small ruminants in Mexico are *Haemonchus contortus*, *Trichostrongylus colubriformis*, *T. axei*, *Teladorsagia* (*Ostertagia*) *circumcincta*, *Cooperia* spp., *Oesophagostomum*, *Trichuris ovis*, *Strongyloides papillosus* and *Bunostomum* sp.\(^8,9\). They generally occur simultaneously, causing clinical symptoms that can vary in severity, depending largely on animal age and nutritional status\(^10\). *Haemonchus contortus* is considered one of the most pathogenic nematodes in sheep and goats due to its hematophagous habits and high prolificacy. Infection with *H. contortus* is known as haemonchosis and results in weight loss, poor appetite, decreased body condition, anemia, weakness, emaciation, edema of lower body regions, susceptibility to other diseases and death in young animals\(^11\).

Diseases from GINs occur in countries with tropical and subtropical climates\(^11\), as well as those with temperate climates\(^12\). No matter where they occur, GINs in small ruminants are the cause of substantial losses due to declines in animal productive potential\(^13\). No study has yet been done on the losses generated by GIN in small ruminants in Mexico. However, based on the US$ 445.10 million dollar losses calculated in a study of the
economic impact of GIN in cattle in Mexico\(^{14}\), it is probable that they also cause significant losses in goat and sheep production.

### Synthetic drugs or anthelmintics

Anthelmintic (AH) drugs are intended for control of livestock parasites. They are classified according to their mode of action: 1) benzimidazoles; 2) imidazothiazoles; and 3) macrocyclic lactones\(^{15}\). Benzimidazoles (BZ) bind to the alpha subunit of the \(\beta\)-tubulin protein, preventing polymerization between the alpha and beta subunits, blocking microtubule formation and causing death in nematodes\(^{16,17}\). Imidazothiazoles (IMZ) act selectively as cholinergic agonists (nicotinic receptors) on the muscle cell membranes of GIN, resulting in muscle contraction and spastic paralysis\(^{16}\). Macrocyclic lactone (ML) molecules bind selectively and irreversibly to the subunits of chlorine ion channels activated by different neurotransmitters (e.g. glutamate), causing hyperpolarization of the muscle or neuronal cell membrane, consequent paralysis of the nematode and its expulsion\(^{18}\).

### Anthelmintic resistance

Anthelmintic resistance (AR) occurs when parasite susceptibility declines vis-à-vis a drug dose that would normally eliminate most parasites\(^{19}\). In Mexico, AR has been reported in sheep herds in the states of Tabasco, Chiapas, Yucatán, Campeche, Tlaxcala, Puebla and Veracruz, and is also known to affect cattle\(^{20,21,22,23}\). Some GINs are known to have developed anthelmintic detoxification mechanisms\(^{24,25}\). In nematodes, AR can alter the target protein, as well as transport xenobiotic molecules such as AH via transmembrane proteins (P-glycoproteins, P-gp), both of which play roles in multi-drug resistance\(^{16}\). In Mexico, changes have been reported in the relative expression of P-gp genes associated with AR in isolates from ivermectin (IVM)-resistant and IVM- susceptible *H. contortus*. This suggests they may function as an effective reference germplasm in the design of study strategies for AR diagnosis and control methods aimed at maintaining drug toxicity in the field and controlling GIN. Resistance develops in response to the interaction between many factors, including GIN population density, treatment time and weather conditions, among others, which influence selection of resistance genes\(^{17,26}\).
Environmental consequences of anthelmintic drug use

Most AH are eliminated in the feces and urine. Some, such as ML, are not fully biotransformed inside the animal and when eliminated into the environment can pose a risk to non-target microorganisms, such as beneficial arthropods\(^\text{27}\) or dung beetles\(^\text{28}\). They can also pollute groundwater and generate significant imbalances in aquifer ecosystems. Macrocyclic lactones such as abamectin are extremely toxic to the planktonic crustacean *Daphnia magna* and highly toxic to other daphnids and fish\(^\text{29}\). When in soils, they can harm beneficial organisms such as arthropods, including flies\(^\text{30}\).

Public health risks from anthelmintic drugs

Excessive use of AH in cattle can contaminate meat, milk and its by-products, constituting a public health risk\(^\text{31,32}\). They are widely used and thus pose a serious threat. For instance, in Ireland almost 60% of dairy herds receive preventative administration of AH\(^\text{33}\), while in Brazil 17.8% of milk samples were reported to contain IVM residues\(^\text{34}\). A study of bulk tank milk in Minas Gerás, Brazil, found it to contain amino-benzimidazoles (55.42%), levamisole (53.57%), avermectins (60.24%), thiabendazole (67.47%), moxidectin (73.49%), triclabendazole (45.78%) and benzimidazoles (6.02%)\(^\text{35}\). Research is still needed in Mexico to quantify AH residues in various products and verify their safety\(^\text{36}\).

Alternative methods for nematode control in livestock

Selective deparasitization (FAMACHA\(^\text{©}\))

The FAMACHA\(^\text{©}\) method is a selective deworming strategy based on degree of animal anemia quantified through the paleness of the lower eye mucus membrane as determined using a reference card. The card shows five colors ranging from intense red to pale or white, representing a 1-to-5 scale, and is used to measure coloration of the mucus membrane\(^\text{37}\). When applied in tandem with body condition measurement, stool-parasitological examination, and fecal egg count (FEC), it helps in developing a deworming criterion\(^\text{38}\). The FAMACHA\(^\text{©}\) method is very useful in identifying the risk of *H. contortus* infection in small ruminants\(^\text{39,40}\), but must be applied by a trained professional.
Grazing management

Under tropical conditions, rotational grazing (RG) involves grazing an area for 3.5 d and then letting it rest for 31 d. This considerably reduces GIN in sheep and goats(41). In India, a decrease in FEC of up to 55.52 % has been reported when using RG in comparison to continuous grazing (CG)(42). Another study reported up to a 48.1 % reduction in the L3 population in feces, as well as better weight gain, in animals under a RG scheme compared to those under CG(43).

Protein diet nutritional strategy

Iso-energy and iso-protein diets have been proven to help prevent and control some parasites(44). The protein and energy levels in diets contribute to controlling GIN, and improve macro- and micronutrient quality and quantity(45), consequently strengthening immunity against nematodes(46).

Using plants with anthelminthic activity

Legumes have high contents of secondary metabolites (e.g. condensed and hydrolysable tannins, flavonoids and other groups of polyphenols) which are an alternative for GIN control(47-50). Some legume species in Mexico have shown efficacy against GIN. For example, in vitro and in vivo studies of Leucaena leucocephala show it to have an AH effect against GIN in cattle(51,52). Other legumes such as acacias contain hydroxycinnamic acid derivatives in their leaves, which exert powerful in vitro ovicidal activity against H. contortus, H. placei and Cooperia punctata(53,54). In an in vivo study using acacia leaves, goats artificially infected with H. contortus and administered 10 % dehydrated leaves in their diet exhibited up to a 70 % reduction in elimination of parasite eggs(55). The pods of Acacia farnesina contain flavonoids such as narigenin 7-O-(6″-galloylglucoside), known to be ovicidal and larvicidal against H. contortus(56). Both L. leucocephala and A. farnesina also constitute protein-rich forages for ruminants(57,58). The nuts of the legume Caesalpinia coriaria exhibit antimicrobial and anthelmintic activity in public health and livestock conditions(59,60,61). Gallic acid and a tannin derivative isolated from C. coriaria fruit were found to exercise an AH effect against GIN eggs in cattle(62). When included in complete diets for sheep and goats, this same fruit was found not to affect intake at a diet inclusion level of 2 % for sheep and 10% for goats(63,64). A bio-directed study of the legume tree Prosopis laevigata identified and isolated the flavonoid isorhamnetin which was found to be a potent in vitro nematicide against H. contortus(65).
Vaccination

An effective alternative treatment for nematodes in ruminants under grazing conditions are antigens (ag) from autochthonous isolates of highly pathogenic nematodes, which can exhibit potential immunoprotective activity\(^{(66)}\). For example, analysis of ag from *Haemonchus* spp. is vital in development of recombinant vaccines against the main GINs\(^{(67)}\). Vaccines against GIN are increasingly sought after as research begins to focus on more sustainable approaches to GIN control\(^{(68)}\). An outgrowth of this research has been the first vaccine (Barbervax) against *H. contortus*, which was derived from surface ag isolated from the intestinal lining of nematodes, and provides partial protection against this pathogen. Another study evaluated the proposed immunization of lambs with a recombinant somatic ag (rHC23) versus *H. contortus*, finding that it reduced egg counts by 70 to 80 %\(^{(69)}\). A separate study using goats infected with *H. contortus* analyzed the efficacy of a protein known as transthyretin, derived from *H. contortus* excretion and secretion products (HcTTR). Two 500 μg doses of recombinant HcTTR reduced FEC by 63.7 % and postmortem parasite load by 66.4 %\(^{(70)}\).

**Genetic selection for resistant animals**

Genetic resistance (GR) is variation in immune response present in a population of animals with the ability to control an infection or disease. It is highly dependent on the adaptive immune response and has a specific origin linked to an ag\(^{(71)}\). Resistance to GIN infections has been reported in various sheep breeds. It is mediated by the adaptive immune response after reinfection with a specific pathogen and is related to the animals’ genetic profile in that it is a trait that can be inherited by offspring from parents\(^{(72)}\). Genetic resistance to GIN is therefore a trait that can be pursued in small ruminant production aimed at controlling this problem. The effects of resistance and resilience in this phenotype against GIN infection can be enhanced in future generations by evaluating and selecting breeds and/or crosses of resistant animals for breeding programs\(^{(71,73,74)}\). Selection of animals with a resistant phenotype requires evaluation and measurement of various standards relating to parasitological, immunological and pathogenicity parameters. These include determination of hpg, body condition, hematocrit percentage, antibody (IgA, IgE) concentrations, and degree of eosinophilia, among others\(^{(71,73,74,75)}\). Once a resistant phenotype has been selected it can function as a reference point for improving progeny resistance in rearing programs. Resistant offspring will harbor fewer adult nematodes, reducing elimination of eggs into the environment and consequently reducing L3 contamination of pastures\(^{(73,74)}\). Lower parasitosis rates in a herd will improve production parameters, potentially lessening dependence on AH use and decreasing AH-caused damage to beneficial organisms in pastures\(^{(72,76,77)}\). In small ruminants, genetic improvement is an alternative medium-term control strategy for GIN
parasitosis. Selection of genetic markers and identification of genomic positions (loci) in the chromosomes linked to a resistant phenotype are vital to understanding the mechanisms of the immune response associated with GIN resistance\textsuperscript{(71,76,77,78)}.

**Biological control**

Nematophagous fungi (NF) are among the principal natural enemies of nematodes. In addition to being saprobes, they are parasites or facultative predators of nematodes\textsuperscript{(79)}. The most promising NF in cattle nematode control is *Duddingtonia flagrans*. This fungus produces a large amount of chlamydospores that can be incorporated into feed, or they can be administered orally to animals in an aqueous suspension\textsuperscript{(80,81,82)}. They pass through the digestive tract and once in the feces they capture nematode larvae and feed on them, reducing their population by 70-90\%\textsuperscript{(82-85)}. Decreasing the larvae population in feces reduces infections and re-infections\textsuperscript{(86)}. Studies by INIFAP researchers have shown this strategy to be highly effective in reducing feces larvae populations in cattle and sheep under different production conditions, and in different regions of Mexico. One example is a study of an organic milk production unit in the Malpaso region of the state of Chiapas\textsuperscript{(80)}. There are currently two products available based on *D. flagrans* chlamydospore formulations: BioWorma in Australia\textsuperscript{(82)}, and Bioverm in Brazil\textsuperscript{(87)}. In Mexico, the CENID-SAI of the INIFAP is currently negotiating an agreement with a company to market a product based on chlamydospores from a Mexican *D. flagrans* strain for livestock applications.

**Comprehensive nematode control**

Adequate GIN control requires an understanding of where nematode parasites are found based on their lifecycle. In livestock they are found principally in three areas. In animals they can be found in the gastrointestinal system as histotrophic larvae (L4), pre-adult stages (L5) and adults, in addition to eggs from females. The feces contain eggs, L1 and L2 (pre-infectious) stages, and the L3 (infectious) stage. Soils and pastures harbor L3. Based on this information a comprehensive control strategy can be developed that focuses on these sites (Figure 1) in which different control tools are applied in a coordinated, synergetic approach for more efficient GIN control.
Figure 1: Diagram representing integrated application of the main gastrointestinal nematode control methods in sheep focused on parasite developmental targets within the endogenous and exogenous phases of the biological cycle.

Conclusions

Scientifically proven control measures exist that are effective in herd-level nematode infection. When implemented in a comprehensive way they can improve animal health and herd productivity, while avoiding excessive AH use. The comprehensive nematode control method also reduces the occurrence AR, contributing to a sustainable approach to nematode control.

Challenges and outlook for nematode control in livestock in Mexico

In the future, parasitologists will face a number of challenges in developing control strategies that move away from widespread AH use. The wide variability in parasite population dynamics largely responds to changes in climate. The spread of AR and resulting progressive inefficacy of AH are a growing threat in livestock production systems. Strategies are needed that block or reverse the adaptive genomic mechanisms behind AR. New immunoprotective ag’s based on recombinant technologies can be explored to improve animal immune system effectiveness. Sustainable
technologies can also play a role in control strategies\(^{(91)}\), especially those involving plants and their metabolites with nematocidal activity\(^{(92,93)}\). Application of NF in nematode control in cattle and small ruminants is promising\(^{(80,81,82)}\). In Mexico, this method needs to be developed to a point where it can be marketed and then promoted to producers. Nanoparticles and metabolites from NF are also promising possibilities that need more extensive research\(^{(94)}\), since they are potentially effective additions to the arsenal of nematode control strategies\(^{(95,96)}\).

**Contributions to the study of nematodiasis in livestock**

Researchers in Mexico have contributed to better understanding and addressing nematode infection in livestock. One area of particular emphasis has been anthelmintic resistance, including the use of molecular tools for identification of resistance marker genes against anthelmintic drugs\(^{(17,21,82)}\). Nematode transcriptomes have also been explored as part of a new perspective on the possible reversal of anthelmintic resistance in parasites, as have genetic and molecular detection of animals resistant to parasites\(^{(78,80)}\). Important research is also being done on plants, and metabolites derived from them, with nematocidal activity against livestock parasites. This has generated data that will help to establish the use of plants with antiparasitic activity in livestock production\(^{(61,66,72)}\). A sustainable method of nematode control in ruminants has been developed using a Mexican strain (FTHO-8) of the NF *Duddingtonia flagrans*, a natural predator of nematodes. Resistance spores, or chlamydospores, from this NF have been incorporated into “cookies” or “pellets” for cattle. When ingested they pass through the digestive tract to the feces where they germinate, colonize the feces and form mycelia traps to capture, kill and feed on nematodes, thus interrupting the biological cycle of nematodes\(^{(92)}\). This is another sustainable method that has been successfully tested under different environmental and animal handling conditions\(^{(87,88,91,93)}\). Cutting-edge research is also in progress on the antiparasitic properties of edible fungi, with promising results such as identification of bioactive metabolites that control nematodes\(^{(97)}\).

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Conflicts of interest

The authors declare no conflict of interest.

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