https://doi.org/10.22319/rmcp.v12s3.5840

Review

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

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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.

Received: 06/11/2020

Accepted: 19/01/2021

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 β-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[27] or dung beetles[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[29]. When in soils, they can harm beneficial organisms such as arthropods, including flies[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[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[33], while in Brazil 17.8 % of milk samples were reported to contain IVM residues[34]. A study of bulk tank milk in Minas Geráis, 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 %)[35]. Research is still needed in Mexico to quantify AH residues in various products and verify their safety[36].

Alternative methods for nematode control in livestock

Selective deparasitization (FAMACHA©)

The FAMACHA© 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[37]. When applied in tandem with body condition measurement, stool-parasitological examination, and fecal egg count (FEC), it helps in developing a deworming criterion[38]. The FAMACHA© method is very useful in identifying the risk of *H. contortus* infection in small ruminants[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[71,76,77,78].

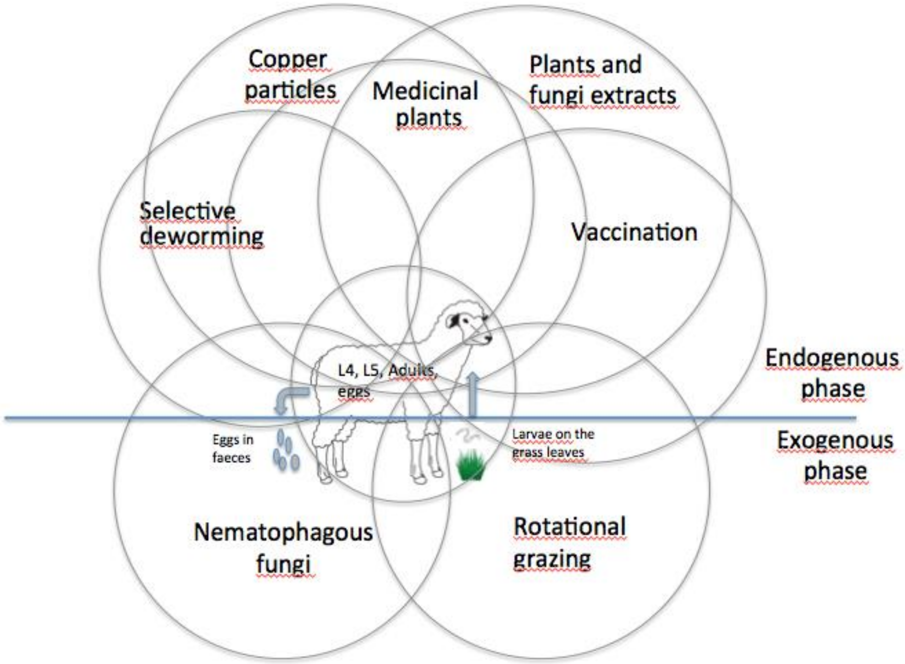
Biological control

Nematophagous fungi (NF) are among the principal natural enemies of nematodes. In addition to being saprobes, they are parasites or facultative predators of nematodes[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[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 %[82-85]. Decreasing the larvae population in feces reduces infections and re-infections[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[80]. There are currently two products available based on *D. flagrans* chlamydospore formulations: BioWorma in Australia[82], and Bioverm in Brazil[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(88). 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(89). New immunoprotective ag´s based on recombinant technologies can be explored to improve animal immune system effectiveness(69,70,90). 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].

Acknowledgments

The authors thank the INIFAP and CONACYT for the support provided our research projects.

Conflicts of interest

The authors declare no conflict of interest.

Literature cited:

1. IICA, Carne Ovina. Caracterización del valor nutricional de alimentos. PROCITUR, IICA, Montevideo, Uruguay; 2015:158-169. http://repiica.iica.int/docs/B3885e/B3885e.pdf.

2. Tuinterfaz. Se logró reducir importaciones en 74%, de 58 mil toneladas a 10 mil 379 toneladas de carne: Sagarpa. 2018. https://tuinterfaz.mx/noticias/22/10454/en-17-anos-la-produccion-de-ovino-crecio-70/.

3. Agnusdei GM. Calidad nutritiva del forraje. Sitio Argentino de Producción Animal. Agromercado Temático 2007, Bs. As., 136:11-17.

4. Díaz-Sánchez CC, Jaramillo-Villanueva JL, Vargas-López S, Delgado-Alvarado A, Hernández-Mendo O, Casiano-Ventura MA. Evaluación de la rentabilidad y competitividad de los sistemas de producción de ovinos en la región de Libres, Puebla. Rev Mex Cienc Pecu 2018;9(2):273-277.

5. Rojas-Downing MM, Nejadhashemi AP, Harrigan T, Woznicki SA. Climate change and livestock: Impacts, adaptation, and mitigation. Clim Risk Managem 2017;16:145–163. doi:10.1016/j.crm.2017.02.001.

6. Craig TM. Gastrointestinal nematodes, diagnosis and control. Vet Clin North Am Food Anim Pract 2018;34(1):185–199. doi:10.1016/j.cvfa.2017.10.008.

7. Roeber F, Jex AR, Gasser RB. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective. Parasit Vectors 2013;6(153). doi:10.1186/1756-3305-6-153.

8. Mondragón-Ancelmo J, Olmedo-Juárez A, Reyes-Guerrero DE, Ramírez-Vargas G, Ariza-Román AE, López-Arellano ME, *et al*. Detection of gastrointestinal nematode populations resistant to albendazole and ivermectin in sheep. Animals 2019;9:775. doi:10.3390/ani9100775.

9. López-Ruvalcava OA, González-Garduño R, Osorio-Arce MM, Aranda-Ibañez A, Díaz-Rivera P. Cargas y especies prevalentes de nematodos gastrointestinales en ovinos de pelo destinados al abasto. Rev Mex Cienc Pecu 2013;4(2):223-234.

10. Schallig HDFH. Immunological responses of sheep to *Haemonchus contortus*. Parasitol 2000;120(7):63–72. doi:10.1017/s003118209900579x.

11. Selemon M. Review on Control of *Haemonchus contortus* in sheep and goat. J Vet Med Res 2018;5(5):1139.

12. Manninen S, Oksanen A. Haemonchosis in a sheep flock in North Finland [2010]. Acta Vet Scand 2010.https://doi.org/10.1186/1751-0147-52-S1-S19.

13. Mavrot F, Hertzberg H, Torgerson P. Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. Parasit Vectors 2015;8:557. https://doi.org/10.1186/s13071-015-1164-z.

14. Rodríguez-Vivas RI, Grisi L, Pérez-de León AA, Silva-Villela H, Torres-Acosta JJJ, Fragoso Sánchez H, *et al*. Potential economic impact assessment for cattle parasites in Mexico. Review. Rev Mex Cienc Pecu 2017;8(1):61-74.

15. Holden-Dye L, Walker RJ. Anthelmintic drugs and nematicides: studies in *Caenorhabditis elegans*. In: The *C. elegans* Research Community (ed.). WormBook; 2014. doi/10.1895/wormbook.1.143.2.

16. Kotze AC, Prichard RK. Antihelmintic resistence in *Haemonchus contortus*: History, mechanisms and diagnosis. In: Gasser RB, Von Samson-Himmelstjerna G. editors. *Haemonchus contortus* and haemonchosis – Past, present and future trends, London: Elsevier Ltd.; 2016:397-428.

17. Lanusse CE, Alvarez LI, Lifschitz AL. Gaining insights into the pharmacology of anthelmintics using *Haemonchus contortus* as model nematode, in: Gasser RB, Von Samson-Himmelstjerna G. editors. *Haemonchus contortus* and haemonchosis – Past, present and future trends, London: Elsevier Ltd; 2016:465–518.

18. Laing R, Gillan V, Devaney E. Ivermectin – old drug, new tricks?, Trends Parasitol 2017;33:463-472. https://doi.org/10.1016/j.pt.2017.02.004.

19. Mphahlele M, Molefe N, Tsotetsi-Khambule A, Oriel T. Anthelmintic resistance in livestock. In: *Helminthiasis*, IntechOpen 2019. http://dx.doi.org/10.5772/intechopen.87124.

20. Medina P, Guevara F, La OM, Ojeda N, Reyes E. Resistencia antihelmíntica en ovinos: una revisión de informes del sureste de México y alternativas disponibles para el control de nematodos gastrointestinales. Pastos y Forrajes 2014;37(3):257-263.

21. Encalada-Mena L, Tuyub-Solis H, Ramírez-Vargas G, Mendoza-de-Gives P, Aguilar-Marcelino L, López-Arellano ME. Phenotypic and genotypic characterisation of *Haemonchus* spp. and other gastrointestinal nematodes resistant to benzimidazole in infected calves from the tropical regions of Campeche State, Mexico, Vet Parasitol 2014;205:246–254. https://doi.org/10.1016/j.vetpar.2014.06.032.

22. González-Garduño R, Torres-Hernández G, López-Arellano ME, Mendoza-de-Gives P. Resistencia antihelmíntica de nematodos parásitos en ovinos, Rev Geogr Agrí 2012;48:63–74. https://www.redalyc.org/articulo.oa?id=75730739005.

23. Alonso-Díaz MA, Arnaud-Ochoa RA, Becerra-Nava R, Torres-Acosta JF, Rodriguez-Vivas RI, Quiroz-Romero RH. Frequency of cattle farms with ivermectin resistant gastrointestinal nematodes in Veracruz, Mexico. Vet Parasitol 2015;212(3-4):439-443.

24. Lindblom TH, Dodd AK. Xenobiotic Detoxification in the nematode *Caenorhabditis elegans*. J Exp Zool 2006;305(9):720-730.

25. Reyes-Guerrero DE, Cedillo-Borda M, Alonso-Morales RA, Alonso-Díaz MA, Olmedo-Juárez A, Mendoza-de-Gives P, *et al*. Comparative study of transcription profiles of the P-glycoprotein transporters of two *Haemonchus contortus* isolates: susceptible and resistant to ivermectin, Mol Biochem 2020;238(111281):1-7. https://doi.org/10.1016/j.molbiopara.2020.111281.

26. Traversa D, von Samson-Himmelstjerna G. 2016. Anthelmintic resistence in sheep gastro-intestinal strongyles in Europe. Small Rum Res 2016;135:75-80.

27. Floate KD, Wardhaugh KG, Boxall AB, Sherratt TN. Fecal residues of veterinary parasiticides: nontarget effects in the pasture environment. Annu Rev Entomol 2005;50:153-179.

28. Verdú JR, Cortez V, Martinez-Pinna J, Ortiz AJ, Lumaret JP, Lobo JM, *et al*. First assessment of the comparative toxicity of ivermectin and moxidectin in adult dung beetles: Sub-lethal symptoms and pre-lethal consequences. Sci Rep 2018;8(14885). doi:10.1038/s41598-018-33241-0.

29. Tišler T, Kožuh-Eržen N. Abamectin in the aquatic environment. Ecotoxicol 2006;15:495–502. https://doi.org/10.1007/s10646-006-0085-1.

30. Daeseleire E, Van Pamel E, Van Poucke C, Croubels S. Veterinary drug residues in foods. In: Schrenk D, editor. Chemical contaminants and residues in food. 1st ed. Woodhead Publishing; 2017:117–153. doi:10.1016/b978-0-08-100674-0.00006-0.

31. Beyene T. Veterinary drug residues in food-animal products: Its risk factors and potential effects on public health. J Vet Sci Tech 2015;07(01):doi:10.4172/2157-7579.1000285.

32. Moreno L, Lanusse C. Veterinary drug residues in meat-related edible tissues. In: Moreno L, Lanusse C. editors. New aspects of meat quality. Elsevier: 2017:581-603. doi:10.1016/b978-0-08-100593-4.00024-2.

33. Bennema SC, Vercruysse J, Morgan E, Stafford K, Hoglund J, Demeler J, *et al*. Epidemiology and risk factors for exposure to gastrointestinal nematodes in dairy herds in northwestern Europe. Vet Parasitol 2010;173:247–254.

34. Lobato V, Rath S. Reves FGR. Occurence of ivermectin in bovine milk from the Brazilian retail market. Food Addit Contam 2006;23:668-673.

35. Cerqueira OPM, Souza NF, França-da-Cunha A, Almeida-Picinin LC, Leite OM, Souza RM, *et al.* Detection of antimicrobial and anthelmintic residues in bulk tank milk from four different mesoregions of Minas Gerais State -Brazil Minas Gerais - Brasil. Arq Bras Med Vet e Zootec 2014;66(2):621-625.

36. Lourenco A, Fraga M, De Colli L, Moloney M, Danaher M, Jordan K. Determination of the presence of pathogens and anthelmintic drugs in raw milk and raw milk cheeses from small scale producers in Ireland. LWT 2020;109347. doi:10.1016/j.lwt.2020.109347.

37. Kaplan RM, Burke JM, Terril TH, Miller JE, Getz WR, Valencia E, *et al.* Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet Parasitol 2004;123(1-2):105-120.

38. Moors E, Gauly M. Is the FAMACHA© chart suitable for every breed? Correlations between FAMACHA©scores and different traits of mucosa colour in naturally parasite infected sheep breeds. Vet Parasitol 2009;166(1-2):108-111.

39. Harlow I. FAMACHA scoring to identify parasite risk in small ruminants. Farm & Dairy 2016. https://www.farmanddairy.com/top-stories/famacha-scoring-to-identify-parasite-risk-in-small-ruminants/316777.html.

40. Gonçalves-da Silva D, Martins de Menezes B, Fernandes Bettencourt A, Frantz AC, Ribeiro-Corrêa M, Ruszkowski G, *et al*. Método FAMACHA® como ferramenta para verificar a infestação parasitária ocasionada por *Haemonchus* spp. em ovinos PubVet 2017;11(10):1015-1021. doi:10.22256/pubvet.v11n10.1015-1021.

41. Barger IA, Siale K, Banks DJD, Le Jambre LF. Rotational grazing for control of gastrointestinal nematodes of goats in a wet tropical environment. Vet Parasitol 1994;53:109–116.

42. Ram-Prasad MS, Sundaram SM, Gnanaraj PT, Bandeswaran C, Harikrishnan TJ, Sivakumar T, *et al.* Influence of intensive rearing and continuous and rotational grazing systems of management on parasitic load of lambs. Vet World 2019;12(8):1188-1194.

43. Devi T, Muthuramalingam T, Tensingh-Gnanaraj P, Bino-Sundar ST, Serma-Saravana-Pandian A, Jemimah R. Rotational grazing pasture management system in sheep in Tamil Nadu to gain better bodyweight through the control of nematodes. J Anim Res 2019;9(3):495-497. doi:10.30954/2277-940X.03.2019.16.

44. Torres-Acosta JFJ, Sandoval-Castro CA, Hoste H, Aguilar-Caballero AJ, Cámara-Sarmiento R, Alonso-Díaz MA. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. Small Ruminant Res 2012;103:28-40.

45. Hoste H, Torres-Acosta JFJ, Quijada J, Chan-Perez I, Dakheel MM, Kommuru DS, *et al.* Interactions Between nutrition and infections with *Haemonchus contortus* and related gastrointestinal nematodes in small ruminants. Adv Parasit 2016;93:239-351. doi:10.1016/bs.apar.2016.02.025

46. Bricarello PA, Amarante AFT, Rocha RA, Cabral Filho SL, Huntley JF, Houdijk JGM, *et al.* Influence of dietary protein supply on resistance to experimental infections with *Haemonchus contortus* in Ile de France and Santa Ines lambs. Vet Parasitol 2005;134:99-109.

47. Lisonbee LD, Villalba JJ, Provenza FD, Hall JO. Tannins and self-medication: Implications for sustainable parasite control in herbivores. Behav Process 2009;82(2):184-189.

48. Williams AR, Ropiak HM, Fryganas C, Desrues O, Muller-Harvey I, Thamsborg SM. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagostomum dentatum*. Parasit Vector 2014;19(7):518. doi: 10.1186/s13071-014-0518-2.

49. Zabré G, Kaboré A, Bayala B, Katiki LM, Costa-Júnior LM, Tamboura HH, *et al*. Comparison of the *in vitro* anthelmintic effects of *Acacia nilotica* and *Acacia raddiana*. Parasite 2017;24(44):1-11. https://doi.org/10.1051/parasite/2017044.

50. Brito DRB, Costa-Júnior LM, Garcia JL, Torres-Acosta JFJ, Louvandini H, Cutrim-Júnior JAA, *et al*. Supplementation with dry *Mimosa caesalpiniifolia* leaves can reduce the *Haemonchus contortus* worm burden of goats. Vet Parasitol 2018;252:47-51.

51. Mejia-Hernández P, Salem AZM, Elghandour MMMY, Cipriano-Salazar M, Cruz-Lagunas B, Camacho LM. Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* Lam. extracts in growing lambs. Trop Anim Health Product 2013;46:173-178.

52. Von Son-de Fernex E, Alonso-Díaz MÁ, Mendoza-de Gives P, Valles-de la Mora B, González-Cortazar M, Zamilpa A, *et al*. Elucidation of *Leucaena leucocephala* anthelmintic-like phytochemicals and the ultrastructural damage generated to eggs of *Cooperia* spp. Vet Parasitol 2015;214(1-2):89–95. doi:10.1016/j.vetpar.2015.10.005.

53. Castillo-Mitre GF, Olmedo-Juárez A, Rojo-Rubio R, Cortázar-González M, Mendoza-de Gives P, Hernández-Beteta EE, *et al*. Caffeoyl and coumaroyl derivatives from *Acacia cochliacantha* exhibit ovicidal activity against *Haemonchus contortus*. J Ethnopharmacol 2017;204:125-131.

54. Olmedo-Juárez A, Rojo-Rubio R, Zamilpa A, Mendoza de Gives P, Arece-García J, López-Arellano ME, *et al*. *In vitro* larvicidal effect of a hydroalcoholic extract from *Acacia cochliacantha* leaf against ruminant parasitic nematodes. Vet Res Commun 2017;41:227-232.

55. Castillo-Mitre GF, Rojo-Rubio R, Olmedo-Juárez A, Mendoza de Gives P, Vázquez-Armijo JF, Zamilpa A, *et al.* El consumo de hojas de *Acacia cochliacantha* reduce la eliminación de huevos de *Haemonchus contortus* en heces de cabritos Boer. Rev Mex Cien Pecu 2021;12(1):138-150.

56. Zarza-Albarrán MA, Olmedo-Juárez A, Rojo-Rubio R, Mendoza-de Gives P, González-Cortazar M, Tapia-Maruri D, *et al*. Galloyl flavonoids from Acacia farnesiana pods possess potent anthelmintic activity against *Haemonchus contortus* eggs and infective larvae. J Ethnopharmacol 2020;249:112402.

57. García-Winder LR, Goñi-Cedeño S, Olguin-Lara PA, Díaz-Salgado G, Arriaga-Jordan CM. Huizache (*Acacia farnesiana*) whole pods (flesh and seeds) as an alternative feed for sheep in Mexico. Trop Anim Health Prod 2009;41:1615–1621.

58. León-Castro Y, Olivares-Pérez J, Rojas-Hernández S, Villa-Mancera A, Valencia-Almazán MT, Hernández-Castro E, *et al.* Effect of three fodder trees on *Haemonchus contortus* control and weight variations in kid. Ecosis Recur Agrop 2015;2(5):193-201.

59 Olmedo-Juárez A, Briones-Robles T, Zaragoza-Bastida A, Zamilpa A, Ojeda-Ramírez D, Mendoza de Gives P, *et al.* Antibacterial activity of compounds isolated from *Caesalpinia coriaria* (Jacq) Willd against important bacteria in public health. Microb Pathog 2019;136:103660.

60. De Jesús-Martínez X, Olmedo-Juárez A, Olivares-Pérez J, Zamilpa A, Mendoza de Gives P, López-Arellano ME, *et al. In vitro* anthelmintic activity of methanolic extract from *Caesalpinia coriaria* J. Willd fruits against *Haemonchus contortus* eggs and infective larvae. Biomed Res Inter 2018;7375693. https://doi.org/10.1155/2018/7375693.

61. De Jesús-Martínez X, Olmedo-Juárez A, Rojas-Hernández S, Zamilpa A, Mendoza-de-Gives P, López-Arellano ME, *et al.* Evaluation of the hydroalcoholic extract elaborated with *Caesalpinia coriaria* Jacq Willd tree fruits in the control of *Haemonchus contortus* Rudolphi. Agrofor Syst 2020;94:1315-1321.

62. García-Hernández C, Rojo-Rubio R, Olmedo-Juárez A, Zamilpa A, Mendoza de Gives P, Antonio-Romo IA, *et al.* Galloyl derivatives from *Caesalpinia coriaria* exhibit *in vitro* ovicidal activity against cattle gastrointestinal parasitic nematodes. Exp Parasitol 2019;200:16-23.

63. Sánchez N, Mendoza GD, Martínez JA, Hernández PA, Camacho-Díaz LM, Lee-Rangel HA, *et al*. Effect of *Caesalpinia coriaria* fruits and soybean oil on finishing lamb performance and meat characteristics. Biomed Res Int 2018;9486258. https://doi.org/10.1155/2018/9486258.

64. García-Hernández C, Olmedo-Juárez A, Mendoza de Gives P, Mondragón-Ancelmo J, Rojo-Rubio R. Efecto nutracéutico del fruto de *Caesalpinia coriaria* (Jacq.) Willd en cabritos infectados artificialmente con *Haemonchus contortus*. En: Memorias de Reunión Anual de Investigación Pecuaria 2019;1:494-496.

65. Delgado-Nuñez EJ, Zamilpa A, González-Cortazar M, Olmedo-Juárez A, Cardoso-Taketa A, Sánchez-Mendoza E, *et al*. Isorhamnetin: A nematocidal flavonoid from *Prosopis laevigata* leaves against *Haemonchus contortus* eggs and larvae. Biomolecules 2020;10:773. doi:10.3390/biom10050773.

66. Bassetto CC, Silva MRL, Newlands GFJ, Smith WD, Ratti Júnior J, Martins CL, *et al*. Vaccination of grazing calves with antigens from the intestinal membranes of *Haemonchus contortus*: effects against natural challenge with *Haemonchus placei* and *Haemonchus similis*. Int J Parasitol 2014;44:697–702. http://dx.doi.org/10.1016/j.ijpara.2014.04.010.

67. Contreras-Ochoa CO, Lagunas-Martínez A, Reyes-Guerrero DE, G.A. Bautista-García G, Tello-López T, González-Garduño R, *et al*. Excreted and secreted products (72/60 kDa) from *Haemonchus placei* larvae induce in vitro peripheral blood mononuclear cell proliferation and actívate the expression of cytokines and FCεR1A receptor. Exp Parasitol 2019;206:1-7. https://doi.org/10.1016/j.exppara.2019.107755.

68. Bassetto CC, Amarante AFT. Vaccination of sheep and cattle against haemonchosis. J Helminthol 2015;doi:10.1017/S0022149X15000279.

69. González-Sánchez ME, Cuquerella M, Alunda JM. Vaccination of lambs against *Haemonchus contortus* with the recombinant rHc23. Effect of adjuvant and antigen dose. PLoS ONE 2018;13(3):e0193118. https://doi.org/10.1371/journal.pone.0193118.

70. Tian X, Lu M, Jia C, Bu Y, Aimulajiang K, Zhang Y, *et al*. *Haemonchus contortus* transthyretin domain - containing protein (HcTTR): a promising vaccine candidate against *Haemonchus contortus* infection. Vet Parasitol 2020;109045. doi:10.1016/j.vetpar.2020.109045

71. Maza-Lopez J, Pacheco-Armenta MJ, Reyes-Guerrero DE, Olmedo-Juárez A, Olazarán-Jenkins S, *et al.* Immune response related to Pelibuey sheep naturally infected with gastrointestinal nematodes in a tropical region of Mexico. Vet Parasitol Regional Stud Rep 2020;21:100422 https://doi.org/10.1016/j.vprsr.2020.100422.

72. Preston SJM, Sandeman M, González J, Piedrafita D. Current status for gastrointestinal nematode diagnosis in small ruminants: Where are we and where are we going? J Immunol Res 2014;210350:1-12. https://doi.org/10.1155/2014/210350.

73. Estrada-Reyes Z, López-Arellano ME, Torres-Acosta F, López-Reyes A, Lagunas-Martínez A, Mendoza-de-Gives P, *et al*. Cytokine and antioxidant gene profiles from peripheral blood mononuclear cells of Pelibuey lambs after *Haemonchus contortus* infection. Parasite Immunol 2017;39(6):e12427. https://doi.org/10.1111/pim.12427.

74. Estrada-Reyes ZM, Tsukahara Y, Amadeu RR, Goetsch AL, Gipson TA, Sahlu T, *et al.* Signatures of selection for resistance to *Haemonchus contortus* in sheep and goats. BMC Genomics 2019;20(735):1-14. https://doi.org/10.1186/s12864-019-6150-y.

75. Reyes-Guerrero DE, López-Arellano ME, González-Garduño R, Ramírez-Vargas G, Mendoza-de-Gives P, Olazarán-Jenkins S, *et al*. Identificación del alelo B del gen de interferón gamma asociado al rechazo de la infección por *Haemonchus contortus* en corderos Pelibuey. Quehacer Científico en Chiapas 2016;11(2):3-9.

76. Hill WG. Is continued genetic improvement of livestock sustainable? Genetics 2016;202:877–881. doi: 10.1534/genetics.115.186650.

77. Schultz B, Serao N, Ross JW. Genetic improvement of livestock, from conventional breeding to biotechnological approaches. In: Bazer FW, *et al*, editors. Animal Agriculture. USA: Academic Press 2020:393-405. https://doi.org/10.1016/B978-0-12-817052-6.00023-9.

78. Sallé G, Moreno C, Boitard S, Ruesche J, Tircazes-Secula A, Bouvier F, *et al*. Functional investigation of a QTL affecting resistance to *Haemonchus contortus* in sheep. Vet Res 2014;45(1):45-68.

79. Nordbring-Hertz B, Jansson HB, Tunlid A. Nematophagous fungi. eLS 2011. doi:10.1002/9780470015902.a0000374.pub3.

80. Ortíz-Pérez DO, Sánchez-Muñoz B, Nahed-Toral J, Orantes-Zebadúa MÁ, Cruz-López JL, Reyes-García ME, *et al*. Using *Duddingtonia flagrans* in calves under an organic milk farm production system in the Mexican tropics. Exp Parasitol 2017;175;74–82.

81. Mendoza-de-Gives P, López-Arellano ME, Aguilar-Marcelino L, Jenkins-Olazarán S, Reyes-Guerrero DE, Ramírez-Vargas G, *et al*. The nematophagous fungus *Duddingtonia flagrans* reduces the gastrointestinal parasitic nematode larvae population in faeces of orally treated calves maintained under tropical conditions. Dose/response assessment. Vet Parasitol 2018;15(263):66-72 doi:10.1016/j.vetpar.2018.10.001.

82. Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kos-Durjava M, *et al.* Scientific Opinion on the safety and efficacy of BioWorma® (*Duddingtonia flagrans* NCIMB 30336) as a feed additive for all grazing animals. EFSA Journal 2020;18(7):6208. doi:10.2903/j.efsa.2020.6208.

83. Llerandi-Juárez RD, Mendoza-de Gives P. Resistance of chlamydospores of nematophagous fungi to the digestive processes of sheep in Mexico. J Helminthol 1998;72:155–158.

84. Mendoza-de Gives P, Flores-Crespo J, Herrera-Rodríguez D, Vázquez-Prats VM, Liébano-Hernández E, Ontiveros-Fernández GE. Biological control of *Haemonchus contortus* infective larvae in ovine faeces by administering an oral suspension of *Duddingtonia flagrans* chlamydospores to sheep. J Helminthol 1998;72:343–347.

85. Casillas-Aguilar JA, Mendoza-de-Gives P, López- Arellano ME, Liébano-Hernández E. Evaluation of multinutritional pellets containing *Duddingtonia flagrans* chlamydospores for the control of ovine haemonchosis. Ann N Y Acad Sci 2008;1149:161–163.

86. Mendoza-de Gives P, Zapata-Nieto C, Liébano-Hernández E, López-Arellano ME, Rodríguez HD, Garduño RG. Biological control of gastrointestinal parasitic nematodes using *Duddingtonia flagrans* in sheep under natural conditions in Mexico. Ann N Y Acad Sci 2006;1081(1):355–359. doi:10.1196/annals.1373.050.

87. Ribeiro-Braga F, Magri-Ferraz C, da Silva NE, de Araújo VJ. Efficiency of the Bioverm (*Duddingtonia flagrans*) fungal formulation to conrol *in vivo* and *in vitro* of *Haemonchus contortus* and *Strongyloides papillosus* in sheep. 3 Biotech 2020;10(62). https://doi.org/10.1007/s13205-019-2042-8.

88. Sallé G, Doyle SR, Cortet J. *et al*. The global diversity of *Haemonchus contortus* is shaped by human intervention and climate. Nat Commun 2019;10(4811). https://doi.org/10.1038/s41467-019-12695-4.

89. Chaudhry U, Redman EM, Kaplan R, Yazwinski T, Sargison N, Gilleard JS. Contrasting patterns of isotype-1 β-tubulin allelic diversity in *Haemonchus contortus* and *Haemonchus placei* in the southern USA are consistent with a model of localised emergence of benzimidazole resistance. Vet Parasitol 2020;109240. doi:10.1016/j.vetpar.2020.109240 https://doi.org/10.1016/j.vetpar.2020.109240.

90. Shamim A, Sajid MK, Imran M, Saqib MN. Peptides isolation from crude somatic antigens of *Haemonchus contortus* through SDS- PAGE. Indian J Ani Res 2017;52(914-916).doi: https://doi.org/10.18805/ijar.v0iOF.8473.

91. Powell K, Foster C, Evans S. Environmental dangers of veterinary antiparasitic agents. Vet Rec 2018;183(19):599–600. doi:10.1136/vr.k4690.

92. Githiori JB, Höglund J, Waller PJ. Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. Anim Health Res Rev 2005;6(01):91–103. doi:10.1079/ahr2005099.

93. Minho PA, Domingues FL, Gainza AY, Figueiredo A, Boligon A, Domingues R, *et al*. *In vitro* screening of plant extract on *Haemonchus contortus and Rhipicephalus (Boophilus) microplus*. J Essential Oil Res 2020. doi: 10.1080/10412905.2020.1746414.

94. Magri-Ferraz C, Pinheiro CSL, Elias-de-Freitas SF, Oliveira-Souza RL, Tobias LF, Victor-de-Araújo J, *et al*. Effect of silver nanoparticles (AgNP's) from Duddingtonia flagranson cyathostomins larvae (subfamily: cyathostominae). J Invertebr Pathol 2020;107395. doi:10.1016/j.jip.2020.107395.

95. Degenkolb T, Vilcinskas A. Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. Appl Microbiol Biot 2016;100(9):3799-3812.

96. Ocampo-Gutiérrez AY, Hernández-Velázquez VM, Aguilar-Marcelino L, Cardoso-Taketa A, Zamilpa A, López-Arellano ME, *et al*. Morphological and molecular characterization, predatory behaviour and effect of organic extracts of four nematophagous fungi from Mexico, Fungal Ecol 2021;49(101004). https://doi.org/10.1016/j.funeco.2020.101004.

97. Cruz-Arévalo J, Sánchez JE, González-Cortazar M, Zamilpa A, Andrade-Gallegos HR, Mendoza-de-Gives P, *et al.* Chemical composition of an anthelmintic fraction of *Pleurotus eryngii* against eggs and infective larvae (L3) of *Haemonchus contortus*. BioMed Res Int Hindawi 2020;2020:4138950. doi: https://doi.org/10.1155/2020/4138950.