UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MÉXICO

CENTRO UNIVERSITARIO UAEM AMECAMECA

DOCTORADO EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

EFECTO DE LA CEBOLLA (*Allium cepa*) EN EL CONTROL DE PARÁSITOS INTESTINALES DIAGNOSTICADOS POR PCR, DE OVINOS DE AMECAMECA

TESIS

QUE PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS AGROPECUARIAS Y RECURSOS NATURALES

PRESENTA:

RAFAEL HEREDIA CARDENAS

Amecameca de Juárez, Estado de México, Marzo, 2018

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DEDICATORIA

« A Nayle, Rafaella, Montserrat y mis Padres »

RESUMEN

Introducción. Los parásitos gastrointestinales son responsables de enormes pérdidas en la producción ovina a nivel mundial, entre los parásitos que infectan a los ovinos se encuentra *Haemonchus contortus,* es un nematodo hematófago responsable de provocar anemia e hipoproteinemia en el hospedador, lo que a su vez disminuye el desarrollo de los animales y los hace susceptibles a otras enfermedades, el control de este nematodo se complica debido a la constante reinfección durante el pastoreo, también por la ausencia de diagnóstico certero con la utilización de microscopia y técnicas moleculares como PCR para la identificación de aislados resistentes, esto aunado un inadecuado manejo de los tratamientos antihelmínticos, estos principalmente han generado resistencia por parte de los parásitos. La utilización de plantas para el control de parásitos es una alternativa que ha reportado tener disminución en el número de huevos por gramo de heces en diferentes grados dependiendo de la sustancia bioactiva presente. La cebolla (*Allium cepa*) es un cultivo consumido ampliamente en México tanto para alimentación como para uso medicinal tradicionalmente. *Allium cepa* contiene compuestos volátiles y no volátiles como tiosulfinatos, sapogeninas, saponinas y flavonoides, a los cuales se les han atribuido propiedades antioxidantes, antimicrobiales, antitumorales y antihelmínticas.

Objetivo. Evaluar el efecto de la cebolla (*Allium cepa*) para el control de *Haemonchus contortus* en ovinos.

Metodología. Se utilizaron 24 ovinos infectados naturalmente, se dividieron en 3 grupos; testigo, ivermectina y cebolla, se tomaron muestras de heces directo del recto por 5 semanas, se analizaron mediante técnica de Faust, PCR y McMaster para la identificación de *Haemonchus contortus* y para calcular el número de huevos por gramo de heces, se tomaron muestras de sangre semanalmente y se determinaron valores hematológicos; hematocrito, proteínas plasmáticas, eritrocitos y leucocitos.

Resultados. La administración de *Allium cepa* en ovinos tuvo eficacia de 50% en comparación con ivermectina que tuvo 75%, no hubo diferencia significativa entre tratamientos, *Allium cepa* no tuvo efectos adversos en los parámetros hematológicos.

Conclusión. La utilización de *Allium cepa* es una alternativa viable ya que no altero los parámetros hematológicos en ovinos y disminuyo el número de huevos por gramo de heces y no contamina el ambiente y no tiene tiempo de retiro. Se deben tener las siguientes consideraciones se debe utilizar siempre y cuando sea adquirido como desecho agrícola debido al alto costo y el productor reciba capacitación para su correcta administración.

Palabras clave: *Hemonchus contortus, Allium cepa,* ovinos, control de parásitos, alternativas, diagnóstico molecular

SUMMARY

Introduction. Gastrointestinal parasites are responsible for enormous losses in sheep production worldwide, among the parasites that infect sheep is *Haemonchus contortus*, is a haematophage nematode responsible for causing anemia and hypoproteinemia in the host, which in turn decreases the development of animals and makes them susceptible to other diseases, the control of this nematode is complicated due to the constant reinfection during grazing, also due to the absence of accurate diagnosis with the use of microscopy and molecular techniques such as PCR for the identification of isolates resistant, this combined an inadequate handling of the anthelmintic treatments, these mainly have generated resistance on the part of the parasites. The use of plants for the control of parasites is an alternative that has been reported to have a decrease in the number of eggs per gram of feces in different degrees depending on the bioactive substance present. Onion (*Allium cepa*) is a crop widely consumed in Mexico for both food and medicinal use traditionally. *Allium cepa* contains volatile and non-volatile compounds such as thiosulfinatos, sapogenins, saponins and flavonoids, to which antioxidant, antimicrobial, antitumor and anthelminthic properties have been attributed.

Objective. To evaluate the effect of onion (*Allium cepa*) for the control of *Haemonchus contortus* in sheep.

Methodology. Twenty-four naturally infected sheep were used, they were divided into 3 groups; control, ivermectin and onion, fecal samples were taken straight from the rectum for 5 weeks, analyzed by Faust, PCR and McMaster for the identification of *Haemonchus contortus* and to calculate the number of eggs per gram of stool, samples were taken from blood weekly and hematological values were determined; hematocrit, plasma proteins, erythrocytes and leukocytes**.**

Results. The administration of *Allium cepa* in sheep had efficacy of 50% compared to ivermectin that had 75%, there was no significant difference between treatments, *Allium cepa* had no adverse effects on the hematological parameters.

Conclusion. The use of *Allium cepa* is a viable alternative because it does not alter the hematological parameters in sheep and decreased the number of eggs per gram of feces and does not pollute the environment and has no withdrawal time. The following considerations must be used, as long as it is acquired as agricultural waste due to the high cost and the producer receives training for its correct administration.

Key words: *Hemonchus contortus, Allium cepa*, sheep, parasite control, alternatives, molecular diagnosis

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PÁGINA

1. INTRODUCCIÓN GENERAL

La actual crisis financiera y las pérdidas agrícolas causadas por parásitos tienen un sustancial impacto en la rentabilidad de la explotación (Roeber *et al.,* 2013). Los animales domésticos se encuentran expuestos a numerosos microorganismos tales como bacterias, virus, rickettsias, mycoplasmas, clamidias, hongos y parásitos. Las parasitosis gastrointestinales son generalmente producidas por helmintos (nemátodos, céstodos) y protozoarios (Rodríguez *et al*., 2001). Los nematodos gastrointestinales (NGI) tienen un impacto negativo en la eficiencia nutricional y la productividad de los ovinos (Torres *et al*., 2012). Estas enfermedades afectan con mayor frecuencia a animales jóvenes en desarrollo, provocando baja ganancia de peso y retraso en el crecimiento. Los animales se debilitan y son susceptibles a contraer enfermedades secundarias que incluso les pueden ocasionar la muerte en casos extremos (González *et al*., 2011).

El nematodo *Haemonchus contortus* ha sido considerado como el de mayor prevalencia mundial y uno de los principales causantes de pérdidas económicas en la producción ovina. En algunos países además del *H. contortus* se ha considerado que *Trichostrongylus colubriformis* también tiene gran importancia por su impacto negativo. Otros géneros de importancia son: *Nematodirus, Oesophagostomum, Cooperia, Strongyloides, Teladorsagia, Chabertia, Bunostomum, Trichuris* y *Dictyocaulus* (López *et al*., 2013).

Las infecciones parasitarias especialmente por nematodos gastrointestinales representan un reto debido a la resistencia desarrollada a la mayoría de los antihelmínticos disponibles (Silveira *et al*., 2012), por lo que se han empleado de forma excesiva los antihelmínticos de amplio espectro ocasionando contaminación, además de que incrementan los costos de producción (Githiori *et al*., 2003; Ramos *et al*., 2016), lo cual ha despertado la necesidad para encontrar alternativas no convencionales para el control de parásitos, por ejemplo, las plantas medicinales se caracterizan por sus propiedades antihelmínticas sobre huevos y larvas de parásitos (Camurça *et al*., 2008; Olivieira *et al*., 2009), por tal motivo existe la necesidad de probar con tratamientos alternativos que controlen las parasitosis (Vieira, 2008; Nery *et al*., 2010) ya que existen evidencias que pueden disminuir la carga de parásitos en animales (Gradé *et al*., 2008; Adamu *et al*., 2010; González *et al*., 2011), tales como: plantas ricas en taninos (Joshi *et al*., 2011; Lopes *et al*., 2016), o aceites esenciales (de Aquino *et al*., 2013). En algunas zonas del centro de México, los ovinocultores aplican de forma empírica tratamientos contra parásitos gastrointestinales; como cebolla (*Allium cepa* L.). La cebolla contiene sustancias azufradas volátiles como los tiosulfinatos, además de compuestos fenólicos de tipo esteroideo (Lanzotti, 2006; Githiori *et al*., 2006) y alicina, compuesto azufrado orgánico (Block, 1985). La alicina ha mostrado efecto antihelmíntico en incubaciones *in vitro* empleando ratones infectados con *Babesia microti* (Salama *et al*., 2014).

Para obtener diagnósticos de la parasitosis gastrointestinal se han utilizado estudios de coprología que ayudan a determinar la efectividad de antihelmínticos (González *et al*., 2011), sin embargo estos métodos a pesar de ser utilizados ampliamente presentan limitaciones, ya que solamente permite identificar las familias y en algunos casos se puede llegar hasta la identificación del género, pero no determina las especies por esto es necesario aplicar nuevas herramientas que provean mayor sensibilidad y especificidad como el PCR (Aktas *et al*., 2005). Los recientes avances en biología molecular han proporcionado medios para identificar huevos de nematodos y / o etapas larvarias con una fiabilidad mucho mayor que las técnicas microscópicas, el primer y el segundo espacio interno transcrito (ITS-1 e ITS-2) del ADN ribosomal (ADNr) han demostrado ser fuentes particularmente útiles de marcadores específicos de especie y / o género para este propósito (Bisset *et al*., 2014).

2. REVISIÓN DE LITERATURA

La pérdida de peso, retraso en el crecimiento y la muerte causada por parásitos gastrointestinales son los principales obstáculos en la producción de pequeños rumiantes (Gradé *et al*., 2008). González *et al*. (2011) realizaron un muestreo de tractos gastrointestinales en un rastro de Tabasco a 242 ovinos y reportan que el 57.4% estaban parasitados con alguna especie de las clases nematoda, trematoda o cestoda. Las prevalencias fueron las siguientes: *Haemonchus contortus* 37%, *Cooperia curticei* 36%, *Trichostongylus colubriformis* 25.2%, *Oesophagostomum columbianum* 11%, *Strongyloides papillosus* 3.3%, *Trichostrongylus axei* 4.1%, *Bunostomum trigonocephallum* 0.8%, *Ostertagia ostertagi* y *Trichuris ovis* en un solo animal, se encontró *Fasciola hepatica* en el hígado de los animales sacrificados provenientes de Tabasco y Chiapas, en donde se localizaron en promedio 37 parásitos adultos en cuatro animales, se aisló *Moniezia expansa* en 6.2% de los animales sacrificados provenientes de Veracruz y Tabasco. Rodriguez *et al*. (2001) analizaron 544 muestras de heces de ovinos en el estado de Yucatán, reportando; la presencia de huevos de *Haemonchus* en el 59% de las muestras, *Trichuris* en el 32.16% y *Strongyloides* en el 23.34%.

En el 2013 López *et al*. reportaron que, de 122 animales para abasto, 47 animales (38.5 %) se encontraban parasitados con alguna especie de la clase nematoda (*H. contortus, C. curticei, T. colubriformis*). De estos últimos el 63 % (30/47) de los animales parasitados sólo tuvo una de las tres especies de nematodos, el 2.1 % (1/47) tuvo la combinación *H. contortus* - *C. curticei*, 4.3 % (2/47) tuvo la combinación *H. contortus* - *T. colubriformis*, 17 % (8/47) tuvo la combinación C. *curticei* - T. *colubriformis* y 12.8 % (6/47) de los animales tuvieron las tres especies.

Rojas *et al*. (2007) realizaron un muestreo a 219 animales en Cuetzala del Progreso, Guerrero. La prevalencia de NGI en el presente estudio fue de 77.63% Los géneros identificados fueron: *Haemonchus* sp., con 32%, *Cooperia* sp., con 30%, *Trichostrongylus* sp., con 17.33% y *Oesophogostomun* sp., con 13.67%. Además, se encontró el género *Strongiloides* sp., en un 7.00%.

Los estudios mencionados anteriormente muestran que en promedio los ovinos en México presentan 58.1% de prevalencia de nematodos gastrointestinales, esto sin duda se ve reflejado en la salud de los ovinos y en pérdidas económicas importantes para el sector que explota esta especie.

2.1. Los nematodos gastrointestinales en ovinos

Las nematodosis gastrointestinales en el ganado ovino son enfermedades parasitarias causadas por diferentes géneros de nematodos que se alojan en el tracto digestivo. Su ciclo biológico es directo, con una fase de vida libre y otra parásita (Quiroz *et al*., 2011).

Dentro de las helmintiasis que más afectan a los rumiantes, se encuentran las provocadas por los nematodos gastrointestinales, los cuales se dividen en dos grandes subclases; Phasmidia y Aphasmidia. (Soulsby, 1982). La subclase Phasmidia se encuentra integrada por las familias: a) Trichostronylidae; a esta familia pertenecen los géneros, *Trichostrongylus*, *Haemonchus*, *Cooperia*, *Nematodirus*, *Mecistocirrus* y *Teladorsagia*. b) Ancylostomidae, a esta familia pertenece el género *Bunostomum*. c) Trichonematidae, esta familia incluye al género *Oesophagostomum*. d) Strongylidae, esta familia se encuentra el género *Chabertia*. e) Rhabditidae, el género representativo es *Strongyloides*.

La subclase Aphasmidia se encuentra representada por la familia Trichuridae, el género característico de esta familia es *Trichuris* (Quiroz *et al*., 2011). El período prepatente es normalmente 2-3 semanas, aunque puede ser más de 6 meses para ciertas especies. Las precipitaciones se consideran el principal factor climático que determina la disponibilidad de larvas de estrongilidos y la transmisión de infección en los animales de pastoreo (Blackie, 2014).

Los principales nematodos que afectan a los pequeños rumiantes (ovejas y cabras) pertenecen al orden *Strongylida*: *H. contortus*, *Te. circumcincta* y *Trichostrongylus* spp. (Roeber *et al*., 2013).

2.1.1. *Haemonchus contortus*

H. contortus ha sido considerado como el de mayor prevalencia mundial y uno de los principales causantes de pérdidas económicas en la producción ovina (López *et al*., 2013) es uno de los más nematodos más fértiles, las hembras son capaces de producir miles de huevos por día (5000 a 10000) (González *et al*., 2013), lo que puede conducir a la rápida contaminación del pasto con larvas. En las ovejas, el período pre-patente de *Haemonchus* es 18-21 días, los gusanos adultos sobreviven en sus huéspedes unos pocos meses. Los principales efectos patógenos son causados por la L4 y adultos, que se alimentan de sangre (hemofílicos) causando la muerte de los corderos y en adultos anemia severa la que por lo general se hace evidente después de dos semanas de la infección (Barrère *et al*., 2013). La enfermedad aguda suele depender de la intensidad de la infección y se asocia con signos de anemia hemorrágica, heces de color oscuro, edema, debilidad, reducción de la producción de lana y la masa muscular o a veces la muerte súbita. A diferencia de muchos otros parásitos gastrointestinales, *H. contortus* no es una causa primaria de diarrea y sus efectos en un rebaño a menudo no son fácilmente detectados por la observación de rutina (Roeber *et al*., 2013).

2.2. Diagnóstico

Los métodos parasitológicos convencionales para diagnosticar las infecciones por nematodos incluyen identificación morfológica de gusanos adultos de hospedadores a la necropsia considerado durante muchos años el estándar de oro, otro método no invasivo es la detección de etapas inmaduras (huevos o larvas) en las heces del hospedador, aunque requiere profesionales altamente capacitados (Budischak *et al*., 2015) debido a que los análisis morfológicos pueden ser engañosos, como en la identificación de *Haemonchus* spp. (Brasil *et al*., 2012) ya que los huevos eliminados en las heces son morfométricamente similares a *Ostertagia* spp. *Trichostrongylus* spp., por lo que la técnica de PCR es la ideal cuando se requiere identificar género y especie.

2.2.1. Diagnóstico molecular

Los recientes avances en biología molecular han proporcionado medios para identificar huevos de nematodos y / o etapas larvarias con una sensibilidad mucho mayor que las técnicas microscópicas, el primer y el segundo espacio interno transcrito (ITS-1 e ITS-2) del ADN ribosomal (ADNr) (Brasil *et al*., 2012) han demostrado ser fuentes particularmente útiles de marcadores específicos para *Haemonchus contortus* (Bisset *et al*., 2014) incluso esta herramienta de diagnóstico permite la identificación de polimorfismo en el gen de β-tubulina isotipo 1 que está asociado con la resistencia a antihelmínticos de aislados de *Haemonchus contortus* (Moura *et al*., 2016).

2.3. Tratamientos antihelmínticos

Los nematodos del ganado son controlados principalmente a través de tratamientos antihelmínticos. Incluso con óptimas dosis y tratamientos estratégicos, este tipo de control es caro y en la mayoría de los casos sólo parcialmente eficaz (Roeber *et al*., 2013). Los antihelmínticos utilizados para controlar a los NGI son clasificados según su modo de acción y se agrupan en tres familias principales de antihelmínticos de amplio espectro: los benzimidazoles (Barrère *et al*., 2013) y pro-benzimidazoles; el imidiazotiazol (levamisol) y tetrahidropirimidinas (pirantel, morantel) y por último las lactonas macrocíclicas (avermectinas y milbemicinas).

2.3.1. Benzimidazoles y Probenzimidazoles

Estas moléculas actúan sobre los microtúbulos de las células intestinales y tegumentarias del parásito y se fijan directamente sobre las moléculas de β-tubulina de los parásitos bloqueando así su polimerización y evitando la formación de los microtúbulos (Borgers y De Nollin, 1975; Borgers *et al*., 1975a; Zintz y Frank, 1982; Mottier y Lanusse, 2001; Mottier *et al*., 2006).

Los benzimidazoles y los probenzimidazoles actúan sobre los NGI y los parásitos respiratorios. Algunos de ellos (fenbendazol, oxfendazol, albendazol) tienen cierta actividad sobre los cestodos (Borgers *et al*., 1975b; Mottier *et al*., 2006) y trematodos (Mottier *et al*., 2006; Meany *et al*., 2007).

2.3.2. Imidiazotiazol y Tetrahidropirimidinas

El imidiazotiazol (levamisol) y las tetrahidropirimidinas (pirantel, morantel), están agrupados en la misma clasificación por que tienen el mismo modo de acción, pero sus moléculas son diferentes. Actúan rápido y selectivamente como antagonista colinérgico sobre receptores nicotínicos y extra sinápticos de las membranas de las células musculares de los nemátodos (Mottier *et al*., 2006). Provocan una contracción muscular al afectar la permeabilidad de las membranas de las células musculares, causando parálisis y muerte del nemátodo.

2.3.3. Lactonas Macrocíclicas

Las lactonas macrocíclicas (LM) son moléculas que paralizan la faringe, los músculos del cuerpo y del útero de los NGI (Vercruysse y Rew, 2002). Las LM actúan aumentando la permeabilidad de los canales de cloro de las células musculares de dichos órganos (Brard y Chartier, 1997). Otra acción de las LM es sobre los receptores antagonistas del ácido gamma aminobutírico que es un neurotransmisor, provocando una hiperpolarización de las membranas somáticas musculares que se traduce en parálisis muscular (Brard y Chartier, 1997; Prichard, 2002).

2.4. Resistencia antihelmíntica

El uso intensivo de los fármacos antihelmínticos para controlar parásitos se ha hecho poco fiable debido al desarrollo de resistencia en los nematodos gastrointestinales (Roeber *et al*., 2013). Además, el alto costo del desarrollo de nuevos medicamentos, el acceso limitado a los antihelmínticos modernos por los agricultores de escasos recursos en los países en desarrollo y el aumento de la conciencia acerca de las consecuencias en el medio ambiente, indican que es necesario desarrollar nuevas estrategias de control no convencional (Saddiqi *et al*., 2010).

La determinación de esta resistencia es de naturaleza genética, medida por un incremento en la frecuencia de expresión de un carácter hereditario que le confiere a ciertos parásitos de una población dicha resistencia con relación a la población susceptible de una misma especie (Mottier y Lanusse, 2001). Para los NGI, estos fenómenos de quimioresistencia fueron conocidos a principios de los años 70. En diferentes países, los casos aislados de resistencia a todas las moléculas actualmente comercializadas comienzan a ser reportadas (Van Wyk *et al*., 1999). La resistencia antihelmíntica (RA) ha sido descrita en la mayoría de las especies de nemátodos de los rumiantes. El *H. contortus* es la especie señalada como la que presenta más el fenómeno de resistencia (Jackson y Coop, 2000; Kaplan, 2004). Muchos factores explican la aparición de la resistencia en el seno de las poblaciones de los parásitos.

Estos factores son ligados principalmente a la modalidad del uso de estos tratamientos que son la primera causa del aumento de la presión de selección (Jackson y Coop, 2000). La frecuencia continua y desmesurada del uso de desparasitantes en las unidades de producción, es una de las primeras causas evocadas por explicar el desarrollo de la resistencia (Kaplan, 2004). Los errores en la utilización de estas moléculas principalmente de benzimidazoles, como la sobre dosis, ha sido igualmente identificada como una causa en la aparición de la resistencia en la población parasitaria (Chartier y Hoste, 2004).

2.5. Métodos alternativos a los tratamientos convencionales

Dada la disminución de la eficacia de las drogas antihelmínticas, muchos esfuerzos han sido desarrollados para encontrar los métodos alternativos de lucha compatible con un control más durable del parasitismo (Larsen, 1999; Chandrawathani *et al*., 2003; Hoste *et al*., 2006; Ketzis *et al*., 2006). Por otra parte, la resistencia a los productos químicos ha hecho crucial la búsqueda de soluciones alternativas o complementarias a los fármacos sintéticos, el uso de plantas con propiedades antihelmínticas se han considerado un método más viable (Vargas *et al*., 2014), estas han sido investigadas por sus propiedades y han demostrado ser eficaces contra parásitos gastrointestinales lo que a largo plazo evitaría la contaminación de suelos (Camurça *et al*., 2008, Bidkar *et al*., 2012).

Allium cepa tiene una larga historia de uso medicinal, las investigaciones muestran que contiene productos químicos conocidos como compuestos orgánicos de azufre y propiedades como antihelmíntico (Sampath-Kumar *et al*., 2011).

2.5.1. Cebolla (*Allium cepa)*

La cebolla (*Allium cepa*) forma parte de la dieta diaria para la mayoría de la población y es un cultivo de gran importancia económica en todo el mundo (Bhattacharjee *et al*., 2013). *Allium cepa* es la segunda hortaliza más importante en el mundo, después de los tomates, con una producción anual de alrededor de 66 millones de toneladas (Bello *et al*., 2013). La primera cita de estas plantas se encuentra en el Codex Ebers (1550AC), un papiro médico egipcio que da informes de varias fórmulas terapéuticas basadas en cebollas como remedio útil para una variedad de enfermedades tales como problemas del corazón, dolor de cabeza, mordeduras, gusanos y tumores (Lanzotti, 2006). Algunos autores le atribuyen propiedades protectoras contra el cáncer, mata hongos y bacterias, promotor de la salud cardiovascular, reduce la hipertensión arterial y la resistencia a la insulina, ayuda en la pérdida de peso, posee actividad antioxidante, auxiliar en la bronquitis crónica, infecciones, fiebre etc. (Campos *et al*., 2003; Ismail *et al*., 2003).

2.5.1.1. Propiedades fisicoquímicas

Se le suele considerar como un vegetal, también tiene una larga historia de uso medicinal, principalmente la bombilla carnosa que crece debajo de la tierra se usa medicinalmente, así como para comida, pero otras partes de la planta también tienen un lugar en las medicinas tradicionales (Sampath-Kumar *et al*., 2011).

El primer análisis de cebolla registrado fue en 1996 con la detección de siete compuestos los flavonoides encontrados fueron: quercetina, quercetina, monoglucósido, diglucósido quercetina, isorhamnetina, isorhamnetina, glucósido, rutina, y kaempferol (Lanzotti, 2006).

2.5.1.2. Propiedades biológicas

En la medicina popular de la India, el bulbo de *Allium cepa* se utiliza para tratar la disentería, fiebre, bronquitis crónica, picaduras de insectos, picaduras, enfermedades de la piel. El bulbo de *Allium cepa* ha demostrado acción disentérica junto con la actividad anti-hongos y antimicrobiana (Bidkar *et al*., 2012). Muchos de estos efectos biológicos están relacionados con los tiosulfinatos, compuestos de azufre volátiles, típicos de las plantas del género *Allium*, entre estos compuestos se encuentran: sapogeninas, saponinas y flavonoides son las clases principales encontradas. Los tiosulfinatos poseen efecto citotóxico y son capaces de matar bacterias hongos y nematodos, Este creciente interés sigue una tendencia general que se orienta hacia el análisis de metabolitos secundarios de los alimentos. Estudios experimentales han proporcionado creciente evidencia de la acción beneficiosa de los flavonoides en múltiples procesos biológicos relacionados con el cáncer (anticarcinógenos, bioactivación, señalización celular, regulación del ciclo celular, angiogénesis, estrés oxidativo e inflamación). Además, los flavonoides, incluyendo quercetina y taxifolina tienen acción en el tracto gastrointestinal por poseer efecto antiulceroso, antiespasmódico y antidiarreico (Lanzotti, 2006).

3. PLANTEAMIENTO DEL PROBLEMA

En los pequeños rumiantes, el parasitismo gastrointestinal se considera como una de las mayores patologías por afectar negativamente la tasa de crecimiento, ocasionando en los animales jóvenes importantes efectos nocivos que repercuten negativamente en el desarrollo de estos y en la economía del productor. En la práctica productiva se ha instaurado la administración regular de antiparasitarios como una rutina que se realiza incontroladamente y sin ningún criterio técnico, lo cual es la principal causa de un aumento de la resistencia de los parásitos. El uso rutinario de antihelmínticos reduce desarrollo de la inmunidad natural contra estos. Las enfermedades parasitarias reducen la producción y la productividad, afectan el comercio local, nacional e internacional e incrementan la pobreza. A nivel biológico lo patógenos compiten por el potencial de producción de los animales y reducen o alteran los subproductos que debieran ser destinados al consumo humano.

4. JUSTIFICACIÓN

El control de parásitos gastrointestinales de los ovinos se ha realizado por más de 40 años, a través del uso de productos químicos comerciales. Sin embargo, en los últimos años se han incrementado los problemas de resistencia en la población de parásitos a nivel mundial, además, han surgido problemas y preocupación por la posible contaminación que surge debido al uso de estos fármacos en la producción animal (residuos en agua, suelo y alimentos). Es por esto que la tendencia de las investigaciones se ha inclinado a buscar alternativas naturales para el control de los parásitos gastrointestinales, entre ellas uso de plantas.

5. HIPOTESIS

El número de huevos por gramo de heces disminuirá en ovinos tratados con *Allium cepa*.

6. OBJETIVOS

6.1. Objetivo general

Evaluar el efecto de la cebolla (*Allium cepa*) para el control de *Haemonchus contortus* en ovinos.

6.2. Objetivos específicos

- Identificar los principales parásitos intestinales de ovinos, mediante análisis coproparasitoscópico a través de técnicas de flotación.
- Estimar el número de huevos por gramo de heces mediante la técnica de Mc Master.
- Identificar *Haemonchus* spp. mediante PCR en heces de ovino.

- Comparar el efecto de *Allium cepa* contra ivermectina para el control de NGI.
- Evaluar parámetros productivos de ovinos tratados *Allium cepa*.
- Evaluar parámetros hematológicos de ovinos tratados *Allium cepa*.

7. MATERIAL Y MÉTODO

El experimento se condujo en la Posta Zootécnica del Centro Universitario UAEM Amecameca de la Universidad Autónoma del Estado de México, bajo la supervisión y aprobación del Comité Académico del Departamento de Ciencia Animal, para el Cuidado y Uso Animal, el cual se encuentra reglamentado por la Ley de Protección Animal del Estado de México, México.

Se utilizaron 24 ovinos machos de 20 kilos de peso de la zona sur oriente del Estado de México, los cuales fueron alimentados con una dieta simulando época de estiaje. Los ovinos fueron alojados en corraletas experimentales individuales, provistas de comederos, bebederos y piso de concreto, siguiendo un diseño completamente al azar con tres tratamientos y ocho repeticiones (ovinos). Los tratamientos antihelmínticos fueron: 1) testigo (no recibió ningún antihelmíntico), 2) inyección subcutánea de 200 mcg/kg de peso vivo (PV) de ivermectina®, 3) tratamiento con 50g de cebolla deshidratada/día/animal, se utilizó la fórmula de Efectividad = (HPG pre-tratamiento – HPG post-tratamiento) /HPG pretratamiento (Vidyashankar *et al*., 2012) para medir la efectividad de los tratamientos midiendo el número de huevos por gramo de heces (HPG) a lo largo del experimento. Los ovinos de los tratamientos testigo, ivermectina recibieron una dieta la cual contenía 60 % de rastrojo de maíz, 35 % de concentrado comercial (14 % PC) y 5 % de melaza, mientras que los que se les proporcionó cebolla deshidratada, se suplió el 5 % de concentrado por la adición de la cebolla.

Prevalencia: Se tomaron muestras de heces directo del recto para el diagnóstico de parásitos intestinales, se analizaron mediante la técnica de flotación con sulfato de zinc y solución saturada Sheather, las muestras positivas fueron analizadas por PCR para su identificación molecular.

La extracción de ADN se realizó directamente de las heces con el kit comercial Extracción DNA ZR Fecal DNA miniprep Zymo Reseach siguiendo las instrucciones del fabricante, se amplifico la región ITS-2 rDNA, utilizando los primers NC1F (5-ACGTCTGGTTCAGGGTTGTT-3) y NC2R (5 TTAGTTTCTTTTCCTCCGCT-3) (Stevenson *et al*., 1995; Brasil *et al*.., 2012). La PCR fue realizada a un volumen de 25 µL, con 8.75 µL H2O libre de nucleasas, 5 µL de Green, 3.0 µL de MgCl2, 1 µL dNTP, 1 µL de cada primer, 0.25 µL de GoTaq DNA polimerasa (Promega) y 5 µL del DNA extraído. Las condiciones para la reacción se dieron mediante un termociclador automático (SimpliAmp™ Thermal Cycler) en base al siguiente protocolo; desnaturalización inicial a 94°C por 2 minutos, seguido de 35 ciclos de desnaturalización a 94°C por 30 segundos, annealing a 54°C por 30 segundos, extensión a 72°C por 1 minuto, con extensión final a 72°C por 10 minutos. Los productos de la PCR (5µ) se visualizaron en geles de agarosa al 2% y se seleccionaron para secuenciación directa. Las secuencias se realizaron en MacroGen, Rockville, USA. Posteriormente las secuencias se analizaron mediante un blast para conocer el porcentaje de identidad y posteriormente se alinearon con secuencias reportadas a nivel mundial.

Cebolla: se obtuvieron bulbos de *Allium cepa* con productores de la zona, se tomaron registros de la fecha de siembra y cosecha, así como del sitio exacto de producción, se identificó y caracterizó la variedad de *Allium cepa*, los bulbos se lavaron con agua destilada y se dejaron secar al aire durante una hora, se retiraron las capas manualmente y se redujeron a trozos pequeños y se secaron en estufa a 25 °C, para luego ser pelletizada y proporcionarse a los ovinos.

Efecto de *Allium cepa* en la carga parasitaria: Se determinó mediante la técnica cuantitativa de Mc Master. Se analizarán las muestras tomadas al inicio, durante y al final del experimento para determinar el efecto de *Allium cepa* en los parásitos encontrados.

Ganancia de peso: los ovinos fueron estabulados y alojados en corraletas individuales, se registró el peso al inicio, durante y al final del experimento y se calculó la ganancia de peso.

Análisis estadístico: Primer momento estadístico: se determinó la distribución de los datos de las variables a evaluar.

Los datos se analizaron mediante la prueba de GLM para hacer comparación entre los 3 grupos, utilizando el software S.A.S.

8. RESULTADOS

8.1. Artículo 1

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RESEARCH ARTICLE
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Evaluation of five treatments to control intestinal parasites in sheep in Ayapango, state of Mexico

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Abstract

Aim: Intestinal parasites are one of the most common problems in sheep production systems. However, the strategies used to eliminate these parasites have not yielded satisfactory results. Therefore, the aim of this study was to determine the effect of five anthelmintics (with different active ingredients) on the parasite load in sheep.

Materials and Methods: In this study, 107 Rambouillet breed sheep were randomly assigned to five groups. Next, fecal samples were taken directly from the rectum and sent to the laboratory for analysis. We then dewormed each group of sheep using different anthelmintic products: Ivermectin 1%/clorsulon 10%, levamisole 12%, closantel sodium 5%, ivermectin 10%, and closantel 5%/albendazole 3.75% with a dosage corresponding to each sheep. At 15 days post-treatment, we took fecal samples and performed a coproparasitoscopic study, using the Faust flotation technique to assess the presence or absence of parasite eggs and the McMaster technique to quantify eggs.

Results: Ivermectin/clorsulon was more effective in eliminating parasites than other anthelmintics used, especially in *Haemonchus* spp.

Conclusion: The results of this study indicate that using ivermectin/clorsulon decreases the number of eggs in feces and is one alternative in controlling parasites in sheep, leading to a reduction in the incidence of health problems, and consequently, improved productivity.

Keywords: anthelmintics, control, intestinal parasites, sheep.

Introduction

Intestinal parasites such as *Haemonchus* spp., *Moniezia* spp., *Ostertagia* spp., and *Chabertia* spp. are one of the most common health problems affecting sheep (especially young animals), and which seriously affect their health, causing poor weight gain, thus making them susceptible to secondary diseases that may eventually cause death [1]. Such problems incur significant economic losses for producers. Grazing management is a factor that predisposes sheep to acquire parasites [2]. The use of anthelmintics in cattle has reduced the impacts of gastrointestinal nematodes [3]. Control strategies involve actions directed to the host, to eliminate parasites and thereby reduce the contamination of pastures [4].

Frequent use of broad spectrum anthelmintics has greatly increased the prevalence of anthelmintic resistance [5], which is why we need studies that provide insight into the types of parasites present in sheep to determine the antiparasitic strategy that should be employed.

In Mexico, products derived from benzimidazole and macrocyclic lactones are the most frequently used in cases of intestinal parasites, given their broad spectrum of action. High parasitic resistance to benzimidazole has been observed, but this is not the case with macrocyclic lactones [6]. Ivermectins are semisynthetic macrocyclic lactones derived from avermectin. Using ivermectin in cattle in Mexico depends on the farmer's and/or veterinarian's perception of the likelihood of parasite infestation of the cattle, which tends to be seasonal [7]. The State of Veracruz, as one of the main cattle producers in the country, consumes large amounts of ivermectin, which could also be the case in other states of México [8]. The constant use of levamisole has led to reduced efficiency (30%) in the control of gastrointestinal nematodes (*Haemonchus contortus* and *Cooperia curticei*) in hair sheep. Moreover, the combination of levamisole with ivermectin is associated with anthelmintic resistance, and closantel/albendazole also leads to resistance, tested using the larval motility test *in vitro* in a study conducted in the state of Chiapas in Mexico [9]. Albendazole, a benzimidazole derivative, is an anthelmintic drug that has been widely used around the world for decades in cattle and small ruminants [3].

These treatments have been selected for use widely by Mexican farmers, sometimes in excessive or inadequate doses. The objective of this study was to evaluate these five treatments to control intestinal parasites in sheep in Ayapango, State of Mexico.

Materials and Methods

Ethical approval

The experiment was approved by Institutional Animal Ethics Committee.

Selection of animals

For this study, 107 Rambouillet breed sheep from the Ayapango, State of Mexico, were randomly selected in the autumn-winter. Specifically, this included 105 females and two males, with an age mean of 21.7 months. The flock was sent out daily for grazing, not separated by fences. Sheep were identified by ear tags, and all sheep had gone more than 8 months without deworming.

Treatments

Sheep were organized into five groups.

Group 1

Consisting of 18 female sheep dewormed with ivermectin 1%/clorsulon 10% at a dosage of 2.0 mg/kg body weight, administered subcutaneously.

Group 2

Consisting of 21 female and one male sheep dewormed with 12% levamisole at a dosage of 6.0 mg/kg, administered via deep intramuscular injection.

Group 3

Consisting of 26 females and one male sheep dewormed with 5% closantel sodium at a dosage of 15 mg/kg, administered orally.

Group 4

Consisting of 18 female sheep dewormed with ivermectin 1% at a dosage of 4.0 mg/kg, administered subcutaneously.

Group 5

Consisting of 22 female sheep dewormed with closantel 5%/albendazole 3.75% at a dosage of 10 mg/kg, administered orally.

The use of a control group was not considered because the five treatments were evaluated and compared according to the difference in the number of eggs pre- and post-treatment.

After application of the treatments, sheep were placed in the same corral, and always had the same diet by grazing together in the same place.

Analysis of fecal samples

From these individuals, we took fecal samples directly from the rectum, which were identified and stored in plastic bags for later analysis. Sample processing was performed in the laboratory of parasitology and microbiology of the Amecameca University Center of the Autonomous University of the State of Mexico, 15 days after the drugs were administered, fecal samples were taken and analyzed by a coproparasitoscopically using the Faust flotation technique with 2.04 g/mol zinc sulfate. McMaster chambers were used for subsequent egg counting and the determination of parasite load [10-12].

Statistical analysis

The results were analyzed using a Kruskal–Wallis non-parametric test because the groups did not have the same number of animals, and the data did not present a normal distribution.

Results and Discussion

Table-1 shows that there was a statistically significant difference ($p<0.0001$) between number of eggs of *Toxocara* spp. before and after administration of 2.0 mg/kg ivermectin/clorsulon. In the case of *Haemonchus* spp., there was also a significant difference ($p=0.0279$) between the number of eggs before and after the same treatment. The same was observed for *Ostertagia* spp. ($p<0.0001$) and *Chabertia* spp. ($p=0.0036$). In contrast, there was no difference in the number of eggs of *Moniezia* spp. ($p=0.1271$) before and after treatment.

In the comparison shown in Table-2, the number of *Ostertagia* spp. eggs before and after treatment showed a statistically significant difference ($p<0.0001$). There was a significant difference in the number of eggs of *Chabertia* spp. ($p<0.0001$) after the administration of 6.0mg/kg of levamisole, while for *Toxocara* spp. there was no significant difference in the number of eggs pre and post-treatment ($p=0.087$). This was also true for *Haemonchus* spp. ($p=0.141$) and *Moniezia* spp. ($p=0.084$).

Table-1: Mean number of eggs pre- and post-treatment with ivermectin/clorsulon in Group 1.

Parasite (eggs)	n	Number of eggs* pre-treatment	Number of eggs* 15 days post-treatment	χ^2	p**
Toxocara spp.	18	25.277	11.722	18.043	<0.0001
Haemonchus spp.	18	21.166	15.833	4.832	0.0279
Moniezia spp.	18	20.111	16.888	2.327	0.1271
Ostertagia spp.	18	25.305	11.694	17.676	<0.0001
Chabertia spp.	18	22.777	14.222	8.450	0.0036

*Mean number of eggs, **p≤0.05

Table-3 shows that treatment with 15 mg/kg closantel sodium resulted in a statistically significant difference in the number of eggs of *Toxocara* spp. ($p<0.0001$), *Ostertagia* spp. ($p<0.0001$), and *Chabertia* spp. ($p<0.0001$), while no statistically significant difference was observed for *Haemonchus* spp. ($p=0.060$) or *Moniezia* spp. ($p=0.1663$).

Table-4 shows there was a statistically significant difference in the number of *Toxocara* spp. ($p=0.0039$), *Haemonchus* spp. ($p=0.0019$), and *Ostertagia* spp. ($p=0.004$) eggs after the administration of 4.0 mg/kg ivermectin, but for *Moniezia* spp. ($p=0.0927$) and *Chabertia* spp. ($p=0.491$), there was no significant difference in egg number.

In the group treated with 10 mg/kg closantel/albendazole (Table-5), there was a statistically significant difference in the *Toxocara* spp. egg number ($p=0.0007$), in contrast to *Cooperia* spp. ($p=0.129$), *Trichostrongylus* spp. ($p=0.177$), *Oesophagostomum* spp. ($p=0.664$), *Haemonchus* spp. ($p=0.263$), *Trichuris* spp. ($p=0.408$), *Moniezia* spp. ($p=0.767$), and *Strongyloides* spp. ($p=0.416$), which showed no differences in egg numbers after treatment.

The closurlon is use for treatment of flukes without effect in nematodes, although this drug combination is used for the treatment of flukes and nematodes, the coproparasitoscopic tests on samples from sheep treated with ivermectin/clorsulon demonstrated efficiency in removing most parasites, especially *Haemonchus* spp., one of the most commonly implicated parasites in weight loss in sheep [13]. The administration of these two drugs had a positive effect

Table-2: Comparison of the number of eggs pre- and post-treatment with levamisole in Group 2.

Parasite (eggs)	n	Number of eggs* pre-treatment	Number of eggs* 15 days post-treatment	χ^2	p**
Toxocara spp.	22	24.727	20.272	2.928	0.087
Haemonchus spp.	22	24.068	20.931	2.161	0.141
Moniezia spp.	22	24.954	20.045	2.983	0.084
Ostertagia spp.	22	31.250	13.750	21.856	<0.0001
Chabertia spp.	22	31.772	13.227	25.820	<0.0001

*Mean number eggs, **$p\leq0.05$

Table-3: Comparison of the number of eggs pre- and post-treatment with closantel sodium in Group 3.

Parasite (eggs)	n	Number of eggs* pre-treatment	Number of eggs* 15 days post-treatment	χ^2	p**
Toxocara spp.	27	35.555	20.444	16.022	<0.0001
Haemonchus spp.	27	30.518	24.481	3.537	0.060
Moniezia spp.	27	29.722	25.277	1.916	0.1663
Ostertagia spp.	27	40.314	14.685	37.314	<0.0001
Chabertia spp.	27	37.611	17.388	24.502	<0.0001

*Mean number of eggs, **$p\leq0.05$

Table-4: Comparison of the number of eggs pre- and post-treatment with ivermectin in Group 4.

Parasite (eggs)	n	Number of eggs* pre-treatment	Number of eggs* 15 days post-treatment	χ^2	p**
Toxocara spp.	18	22.0	15.0	8.352	0.0039
Haemonchus spp.	18	23.527	13.472	9.628	0.0019
Moniezia spp.	18	20.416	16.583	2.827	0.0927
Ostertagia spp.	18	23.222	13.777	8.127	0.004
Chabertia spp.	18	19.333	17.666	0.472	0.491

*Mean number of eggs, **$p\leq0.05$

Table-5: Comparison of the number of eggs pre- and post-treatment with closantel/albendazole in Group 5.

Parasite (eggs)	n	Number of eggs* pre-treatment	Number of eggs* 15 days post-treatment	χ^2	p**
Toxocara spp.	22	26.714	17.500	11.441	0.0007
Cooperia spp.	22	24.857	19.272	2.295	0.129
Trichostrongylus spp.	22	24.523	19.590	1.817	0.177
Oesophagostomum spp.	22	22.761	21.272	0.188	0.664
Haemonchus spp.	22	24.095	20.000	1.251	0.263
Trichuris spp.	22	23.523	20.545	0.684	0.408
Moniezia spp.	22	22.428	21.590	0.087	0.767
Strongyloides spp.	22	23.404	20.659	0.659	0.416

*Mean number of eggs, **$p\leq0.05$

on sheep by reducing the number of various intestinal parasites [14]. In a study in rats infected with *Fasciola*, by Sibille *et al.*, they found that the combination of these anthelmintics in male mice had little efficacy unlike females where efficacy was higher, but in general, if there were flukes reduction post-treatment [15].

Sakhawat *et al.*, in 1997, they reported that levamisole had 100% efficiency that no nematode eggs were recovered from animals treated [16]. In more recent studies have shown that levamisole administration in sheep does not reduce the majority of intestinal parasites [17]. *Haemonchus* spp. has developed resistance due to the regular use of this anthelmintic in sheep [18], this is consistent with our results because the number of eggs of *Toxocara* spp., *Haemonchus* spp., and *Moniezia* spp. not decreased, with this treatment, although the number of eggs to *Ostertagia* spp. and *Chabertia* spp. decreased significantly, which means that, the parasites aforementioned (*Toxocara* spp., *Haemonchus* spp. and *Moniezia* spp.) they were resistant to this drug.

Closantel sodium has efficacy against strongyle populations which are resistant to ivermectin [19], Similarly, the results to this study, as can be seen in Table-3 where we see that; *Toxocara* spp., *Ostertagia* spp., *Chabertia* spp., if they decrease significantly, but it is not very useful against *Haemonchus* spp. as describe Jabbar *et al*., in 2013 [20].

In this research ivermectin shows excellent nematicide efficacy, which is consistent with that reported con Muñoz *et al*., but also it mentions that, this has diminished over time, especially in the genera *Trichostrongylus* and *Haemonchu*s spp., which have developed resistance [21]. This can happen for various reasons, including the application of inadequate doses of ivermectin, the absence of a timetable for deworming according to the cycles of the parasites and their seasonality, as well as the introduction of animals from areas where resistance has occurred [22].

In our results find that a combination of closantel/albendazole, it was only effective against *Toxocara* spp. and it had no effect on *Cooperia* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Haemonchus* spp., *Trichuris* spp., *Moniezia* spp. and *Strongyloides* spp. in our study, which coincides with that reported by other authors who mention that; closantel has demonstrated ineffectiveness in deworming programs, which is why it is suggested to alternate it with other anthelmintics to improve its effect [23]. Meanwhile, albendazole has a prolonged effect and it is most effective when treatment is administered for an extended period [24]. Thus, even in combination, these two anthelmintics are not a good alternative for deworming.

Conclusion

Misuse and uncontrolled anthelmintic administration in sheep production systems have led to the development of resistance in some parasites. Therefore, it is important to monitor this problem and parasite infestation triggers nutritional, health, and economic problems for producers. The results of this study show that effective control of intestinal parasites in sheep can be achieved using a combination ivermectin and clorsulon, which reduces the incidence of intestinal parasitosis.

Authors' Contributions

CR and EA planned and designed the study. EA collected samples. EA analyzed samples. LG, RH, and GM analyzed the data and provided technical support. EA and RH prepared the manuscript with guidance from the other authors. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

References

1. González, G.R., Córdova, P.C., Torres, H.G. and Mendoza, G.P. (2011) Prevalence of gastrointestinal parasites in sheep slaughtered in a trail of Tabasco, Mexico . *Vet. Mex.,* 42: 125-127.
2. Rojas, H.S., Gutiérrez, S.I., Olivares, P.J. and Valencia, A.M.T. (2007) Prevalence of gastrointestinal nematodes in sheep grazing on the top of the MPIO. Cuetzala del Progreso, Guerrero-Mexico. *Rev. Electrón. Vet.,* 8: 1695-7504.
3. Kabaka, W.M., Kitala, P.M., Gitau, G.K., Maingi, N. and Van Leeuwen, J.A. (2012) The efficacy of albendazole and moxidectin in the control of nematode infection in dairy cattle. *Bull. Anim. Health Prod. Afr*., 60: 393-397.
4. Barrios, M., Sandoval, E., Carrillo, H., Dominguez, L. and Marquez, O. (2010) Evaluation of the effect of two forms of oral administration of fenbendazole on the elimination of gastrointestinal nematodes eggs and weight gain in calves double purpose. *Rev. Electrón. Vet.,* 11: 1695-7504.
5. Moore, D.A., Terrill, T.H., Kouakou, B., Shaik, S.A., Mosjidis, J.A., Miller, J.E., Vanguru, M., Kannan, G. and Burke, J.M. (2008) The effects of feeding *Sericea lespedeza* hay on growth rate of goats naturally infected with gastrointestinal nematodes. *J. Anim. Sci.,* 86: 2328-2337.
6. Montalvo, A.X., López, A.M.E., Vázquez, P.V., Liébano, H.E. and Mendoza, G.P. (2006) Anthelmintic resistance of gastrointestinal nematodes in sheep to fenbendazole and ivermectin in the northwest region of the state of Tlaxcala. *Téc. Pecu. Mex.,* 44: 81-90.
7. Solis, R.C., Wilcock, A., Arellano, C.S., Morales, L.A. and McEwen, A.S. (2011) Residues in cattle slaughtered in federally inspected abattoirs in Nuevo Leon, Mexico. *Food Prot. Trends,* 31: 212-215.
8. Cruz, R.M., Martínez, M.I., López-Collado, J.,

Vargas-Mendoza, M., González-Hernández, H. and Fajersson, P. (2012) Effect of ivermectin on the survival and fecundity of *Euoniticellus intermedius* (*Coleoptera*: *Scarabaeidae*). *Rev. Biol. Trop.*, 60: 333-345.
9. González-Garduño, R., López-Arellano, M.E., Ojeda-Robertos, N.E., Liébano-Hernández, E. and Mendoza-de Gives, P. (2014) *In vitro* and field diagnosis of anthelmintic resistance in gastrointestinal nematodes of small ruminants. *Arch. Med. Vet.*, 46: 399-405.
10. Pajuelo, C.G., Luján, R.D., Paredes, P.B. and Tello, C.R. (2006) Application of the technique of spontaneous tube sedimentation diagnostic hookworms. *Rev. Biomed.*, 17: 96-101.
11. Sánchez, M.R.M., Gómez, N.M. and Alva, E.S.I. (2000) Program quality assessment among laboratories. XXVI. The diversity of coproparasitoscopic techniques and quality. *Lab. Acta*, 12: 139-143.
12. Sandoval, E., Morales, G., Ybarra, N., Barrios, M. and Borges, J. (2011) Comparison between two different models of McMaster cameras used for stool samples counting in the diagnosis of infections by gastrointestinal nematodes in ruminants. *Zootec. Trop.*, 4: 495-501.
13. Mireles, M.E.J., Valencia, A.M.T. and Gutiérrez, S.I. (2009) Natural gastrointestinal parasitosis and daily weight gain of infants lambs in the dry tropic of Guerrero, Mexico. *Rev. Electrón. Vet.*, 11: 1695-7504.
14. Martínez, M.I., Cruz, R.M. and Pierre, L.J. (2000) Effect of different pasture management and livestock on dung beetles *Ataenius apicalishinton* and *Ataenius sculptor* Harold (*Scarabaeidae*: *Aphodiinae*: *Eupariini*). *Acta Zool. Mex.*, 80: 185-196.
15. Sibille, P., Calleja, C., Carreras, F., Bigot, K., Galtier, P. and Boulard, C. (2000) *Fasciola hepatica*: Influence of gender and liver biotransformations on flukicide treatment efficacy of rats infested and cured with either clorsulon/ivermectin or triclabendazole. *Exp. Parasitol.*, 94: 227-237.
16. Sakhawat, A., Anwar, A.H.B., Hayat, Z.I. and Hayat, S. (1997) Field evaluation of anthelmintic efficacy of levamisole, albendazole, ivermectin and morantel tartrate against gastrointestinal nematodes of sheep. *Pak. Vet.*, 17: 114-116.
17. Rimbaud, E., Zúñiga, P., Doña, M., Pineda, N., Luna, L., Rivera, G., Molina, L., Gutiérrez, J. and Venegas, J. (2005) First diagnosis of resistance to macrocyclic lactones levamisole and gastrointestinal parasitic nematodes in sheep in Nicaragua. *Rev. Electrón. Vet.*, 6: 1695-7504.
18. Torres, A.J.F., Villarroel, A.M.S., Rodríguez, A.F., Gutiérrez, S.I. and Alonso, D.M.A. (2003) Diagnosis of gastrointestinal nematodes resistant to benzimidazoles and imidazothiazoles in a goat herd Yucatan, Mexico. *Rev. Biomed.*, 14: 75-81.
19. Arece, J., Rodríguez, D.J.G. and Olivares, J.L. (2008) Closantil effectiveness of 5% ® against gastrointestinal strongyles of sheep. *Rev. Salud Anim.*, 30: 59-62.
20. Jabbar, A., Campbell, J.D.A., Charles, A.J. and Gasser, B.R. (2013) First report of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. *Parasit. Vectors*, 6: 243.
21. Muñoz, A.J., Angulo, C.F., Ramírez, R., Vale, O.O., Chacín, E., Simoes, D. and Atencio, A. (2008) Anthelmintic efficacy of doramectin 1% ivermectin 1% and ricobendazol 15% against gastrointestinal nematodes of hair sheep. *Rev. Cient. FCV-LUZ.*, 18: 12-16.
22. Mamani, L.L.W. and Cayo, R.F. (2009) Determination of anthelmintic resistance (*Moniezia expansa, Moniezia benedeni* and *Thysanosoma actioides*) against albendazole and fenbendazole in sheep in three herds of La Paz-Bolivia. *Rev. Electrón. Vet.*,10: 1695-7504.
23. Vilmaris, M., Rodríguez, D.J.G., Alfonso, P., Martín, J., Mengana, E., Pérez, E., Moya, S. and Matos, K. (2011) antiparasitic efficacy of ivermectin and closantel against *Oestrus ovis in naturally infected sheep. Rev. Salud Anim.*, 3: 184-189.
24. Torrelio, A., Vino, L., Mamani, L.W. and Loza, M.M. (2011) Determination of the antihelmintic efficacy of albendazole and fenbendazole in *Moniezia expanza* (Rudolphi 1810) and *Thysanosoma actinioides* (Diesing 1834) (*Cestoda*: *Anoplocephalidae*) in creole sheep infected naturally in a stay of the community of Comanche, county Pacajes department of the la Paz, Bolivia. *J. Selva Andina Res. Soc.*, 2: 2-16.

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8.2. Artículo 2

Anthelmintic Effects of Dehydrated Onion (*Allium cepa* L.) and Tequesquite (Natron of Mexican Texcoco Lakes) in Growing Lambs

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Gastrointestinal parasites affect the health of sheep and in turn produce massive economic losses. To control this problem, different drugs have been used that caused other types of problems, such as resistance of parasites, environmental pollution, or the death of the edaphic fauna. It is therefore crucial to find alternatives to reduce the impacts of parasitic diseases and to minimize the environmental impacts of the chemical drugs.

The study was conducted in the summer season; 32 creole lambs were housed to compare natural additives with anthelmintic activity and a conventional treatment; tequesquite, onion and ivermectin. Over a period of 35 days, the animals received a maintenance diet. We determined daily weight gain, feed conversion, parasitic load, hematocrit and plasma protein levels, as well as leukocyte and erythrocyte counts. The results were analyzed as a completely randomized design.

The parasites with the highest prevalence were *Haemonchus* spp. and *Eimeria* spp. (71.8%), there were no changes ($P > 0.05$) in the productive performance or hematological parameters; however, tequesquite reduced the presence of eggs ($P = 0.03$). The natural

treatments had no adverse effects on the productive and hematological parameters in lambs fed a maintenance diet.

1. Introduction

Worldwide, sheep production is negatively affected by parasites, decreasing health, animal welfare, growth rates, and, consequently, economic viability [1,2,3], mainly in grazing systems [4]. The presence of gastrointestinal parasites in these ruminants modifies digestion and absorption of nutrients [5], resulting in an excessive use of broad-spectrum anthelmintics and the consequent resistance in the parasites, combined with increasing production costs [6, 7]. Therefore, it is crucial to find alternative treatments for parasitosis control [1, 8], such as the use of plant extracts, which can decrease the parasitic loads in animals [9,10,11]; the most widely used substances are extracts of plants rich in tannins [2,12,13] or essential oils [14].

In some areas of central Mexico, sheep farmers empirically apply alternative treatments against gastrointestinal parasites, such as onion (*Allium cepa* L.) and tequesquite (alkaline salts of Lake Texcoco, Mexico). Onions contain volatile sulfur substances such as thiosulfinates, in addition to phenolic compounds of the steroid type [15,16], as well as allicin, an organic sulfur compound [17]. Allicin has shown an anthelmintic effect in *in vitro* assays using mice infected with *Babesia microti* [18]. Sheep producers traditionally also use tequesquite, which is an alkaline rock composed of several macro- and micro-minerals such as Ca, P, Na, K, Fe, Cu, Mg, and Zn [19]. Typically, a tequesquite stone is added to the sheep drinkers as the only treatment against gastrointestinal parasites, but there is no scientific evidence of the effectiveness of this practice. In this context, the objective of this study is to evaluate the anthelmintic effects of dehydrated onion (*Allium cepa* L.) and tequesquite (Mexican natron) in lambs.

2. Materials and Methods

2.1. Study Area and Ethical Clearance. The experiment was conducted in the Experimental Farm of the University Department UAEM Amecameca of the Autonomous University of Mexico State, under the supervision and approval of the Academic Committee of the Department of Animal Science for Animal Care and Use, which is regulated by the Animal Protection Law of the State of Mexico, Mexico.

2.2. Study Population and Study Design. Thirty-two male creole lambs were used, with an initial live weight of 23.28 ± 4.19 kg; the animals were obtained from grazing systems (native pastures) and were naturally infected with enteroparasites. The lambs were housed in individual experimental cages with feeders, drinking troughs, and concrete floors, following a completely randomized design with four treatments and eight replicates (lambs). The anthelmintic treatments were as follows: 1) control (did not receive any treatment), 2) subcutaneous injection of 0.2 μg/kg of live weight (LW) of ivermectin®, 3) treatment with 50 g dehydrated onion/day/animal, and 4) treatment with 200 g of tequesquite in the drinking water. The measure the effectiveness of the treatments via the number of eggs per gram of feces (EPG), we used the following formula:

Effectiveness = (EPG pre-treatment – EPG post-treatment)/EPG pre-treatment [20].

2.3. Diet and Treatments. The sheep of the control, ivermectin, and tequesquite treatments received a diet consisting of 60% corn stubble, 35% commercial concentrate (14% crude protein), and 5% molasses, while the treatment "onion" the diet consisting of 60% corn stubble, 30% commercial concentrate, and 5% dehydrated onions in pellets (Table 1). The diets were analyzed for dry matter, organic matter, and crude protein, using the AOAC procedure [21]. Neutral and acid detergent fiber concentrations (Table 1) were determined according to Van Soest *et al*. [22]. We used the onion variety "bello blanco", harvested in the spring-summer season (May to June); onions were washed, defoliated, dried at room temperature, and oven-dried at30°C. After dehydration, the onions were triturated and pellets were prepared from the concentrate and the same chemical analyzes as described above were performed. Digestibility was determined via *in situ* degradation [23] for 24 hours in 5 x 10 cm polyester bags with a pore diameter of 52 ± 10 μm. The pockets contained 5.0 g of dehydrated onion and were placed in triplicate inside the rumen of a rumen-cannulated Holstein cow receiving the control diet. After incubation, the bags were removed, washed, and dried for 24 hours at 65°C in a forced air oven to determine dry matter degradation. Tequesquite was analyzed by the qualitative method of X-ray fluorescence (FRX) analysis, determining the minerals and compounds [24].

Table 1. Experimental diets for growing lambs using onion (*Allium cepa* L.) and tequesquite

Ingredients %	Control/Ivermenctin/Tequesquite	Onion
Commercial concentrate*	35.00	30.00
Molasses*	5.00	5.00
Corn stubble*	60.00	60.00
Onion*	---	5.00
Chemical composition		
Dry matter %	71.65	77.37
Organic matter**	65.87	71.78
Crude protein**	7.54	7.87
Neutral detergent fiber**	50.67	50.19
Acid detergent fiber**	26.41	27.05

*The ingredients are expressed as-feed basis percentage **Expressed as percentage of dry matter.

2.4. Sampling Technique. The lambs were observed over a period of 35 days, including an adaptation period of 7 days; feed was provided twice daily (9:00 and 18:00). Water was offered *ad libitum* in drinking troughs. Over the course of the experiment, the animals were weighed once a week in the morning to determine daily weight gain; the difference between the offered and the rejected food was determined to assess water and dry food consumption [25] in order to calculate estimated feed conversion. Excreta samples were collected directly from the rectum of each lamb at 0, 7, 14, 21, and 28 days of the experiment to determine the number of eggs per gram of feces (EPG), using the McMaster technique [26]. On the same sampling days, blood was obtained from the jugular vein for hematological analysis [27].

2.5. Data Analysis. The results were analyzed using a completely randomized design ($Yij = \mu + \tau i + \varepsilon ij$; where Yij is the dependent variable, μ is the overall mean, τi is the fixed effect of treatment and εij is the random error), where each lamb was considered an experimental unit and treatments were considered as fixed effects corroborating the normality of the data, using the JMP program [28]. For final weight and parasitic load initial body weight and initial parasitic load were used as covariate using the model $Yij = \mu + \tau i + \beta (Xij - \overline{X}) + \varepsilon ij$, where β is the regression coefficient associated with the covariate deviated from its mean and Xij is the covariate. A comparison of means was performed using Tukey´s test, with a significance level of $P < 0.05$ [29].

3. Results

The chemical composition of the alternative anthelmintic additives, onion and tequesquite, is shown in Table 2. Based on the results of the X-ray fluorescence analysis, tequesquite contains 34.1% potassium carbonate, 30.3% trone $[Na_3H(CO_3)2x2H_2O]$, 30.3% halite

[NaCl], 5.2% biotite [$K(Mg, Fe)3AlSi_3O_{10}(OH, F)_2$], and the trace contents Al, Si, P, S, Cl, Ka, Ti, Cr, As, Pb, Mn, Fe, Ni, Cu, Zn, Br, and Sr.

Table 2. Chemical composition of the onion (*Allium cepa* L.) and tequesquite used as anthelmintics for growing sheep

Ítems	Onion	Tequesquite
Dry matter, %	83.98	85.46
Organic matter*	77.30	10.97
Protein*	0.42	-
Neutral detergent fiber*	16.04	-
Acid detergent fiber*	12.75	-
In-situ rumen digestibility 24 h, %	55.28	-

*Expressed as percentage of dry matter.

In the initial coproparasitoscopic analysis, we identified the following parasites: *Eimeria* spp. 71.8%, *Haemonchus* spp. 71.8%, *Moniezia* spp. 65.6%, *Strongyloides* spp. 43.7%, *Ostertagia* spp. 38.1%, *Trichuris* spp. 31.2%, and *Cooperia* spp. 22.5%; all sheep hosted one or more of the parasites simultaneously.

Table 3. Lamb performance and egg count per gram of feces

Item	Control	Onion	Tequesquite	Ivermectin	SEM	P
Initial weight, kg	21.51	26.04	22.49	22.30	1.26	0.07
Final weight, kg	23.36	27.87	24.56	25.22	1.47	0.19
Daily weight gain, g/d	66	63	75	105	0.02	0.47
Food consumption, g/d	843	911	905	848	0.06	0.79
Water consumption, l/d	1.61	1.31	1.79	1.89	0.18	0.15
Feed Conversion	28.28	16.02	8.76	9.47	6.82	0.18

SEM = standard error of mean, Significant difference $P < 0.05$.

Regarding the productive behavior of lambs (Table 3), no significant differences ($P < 0.05$) were found in terms of initial and final weight, daily weight gain, feed intake, water consumption, and feed conversion. The treatments did not change the parasite load per week (0, 7, 14, 21, 28 days), although the addition of onion, ivermectin, and tequesquite reduced the load to less than half (Table 3). The treatments did not significantly differ ($P < 0.05$) in terms of initial and final values of the number of eggs per gram of feces (EPG); however, in lambs consuming tequesquite, the number of EPG decreased ($P = 0.03$) by 75.70% (Table 4). In terms of the effectiveness (percentage of egg reduction) of the treatments, the onion group obtained 50% effectiveness, tequesquite 75%, and ivermectin 73%.

Table 4. Parasitic load - eggs per gram of initial and final stool per treatment

	Control	Onion	Tequesquite	Ivermectin
Initial (EPG)	4971	5050	7100^{a}	4963
Final (EPG)	4163	2500	1725^{b}	1300
SEM	1401.76	1129.10	1280.29	1049.15
P	0.87	0.27	0.03	0.79

^{ab}Means with different letters within a column are significantly different at $P < 0.05$; EPG= eggs per gram of feces; SEM=standard error of mean.

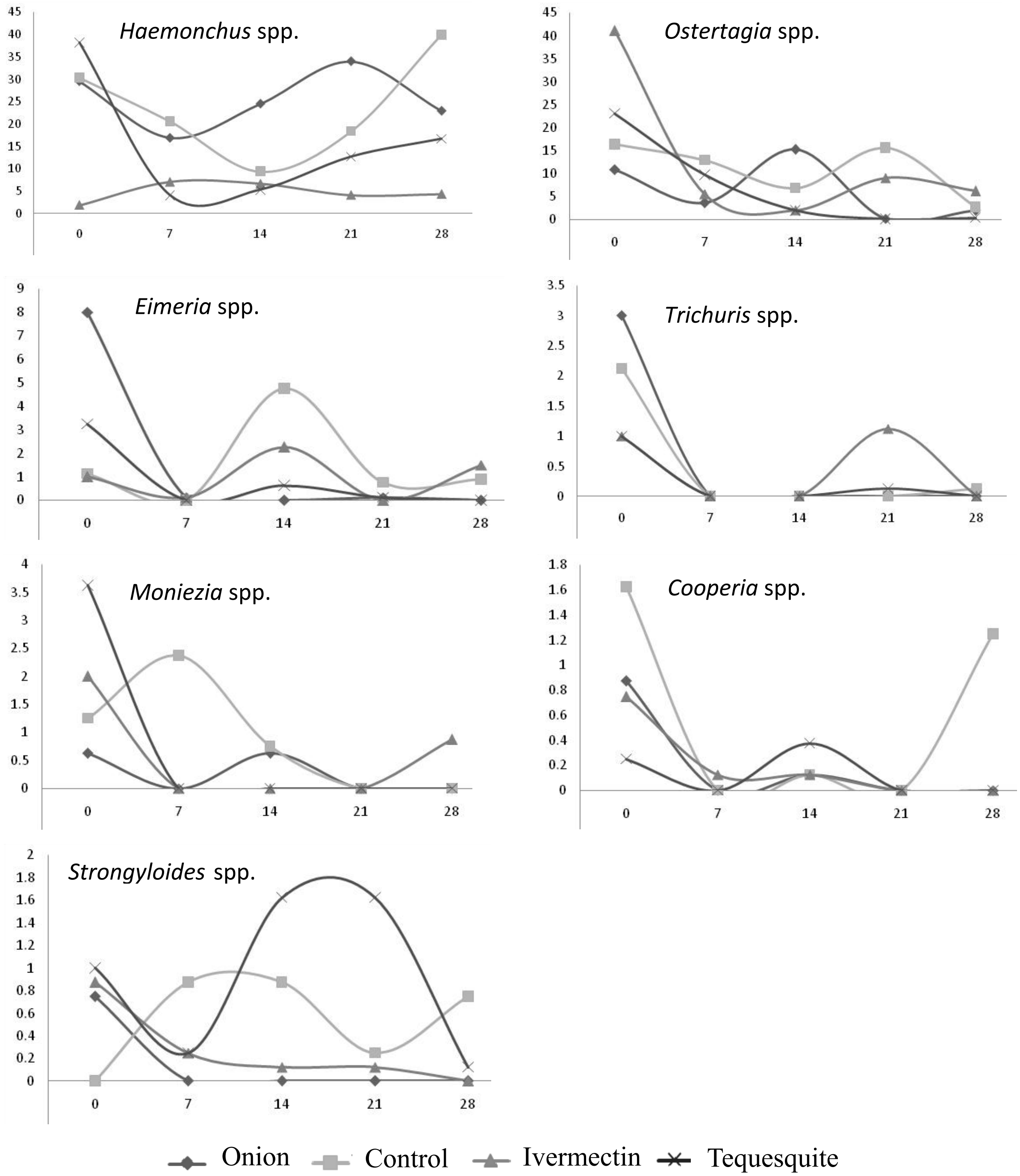

Figure 1. Number of eggs per gram of feces per parasite and treatment during the experiment (0, 7, 14, 21, 28 days), on the vertical axis the number per gram of feces is presented (multiply by 100 to obtain the total value)

Based on the effects of the anthelmintic treatments (Fig. 1), the inclusion of tequesquite and onion decreased the number of eggs per gram of feces at the end of the sampling period in six species of parasites (*Eimeria* spp., *Moniezia* spp., *Ostertagia* spp., *Trichuris* spp.), except for *Haemonchus* spp., *Cooperia* spp., and *Strongyloides* spp., which were kept constant with the use of ivermectin.

There were no significant effects on initial hematological parameters ($P < 0.05$) at seven days after the treatment (Table 5); however, at the end of the trial (day 28), the plasma protein parameters ($P = 0.05$) were modified, as were the leukocyte and erythrocyte counts ($P > 0.01$). (Table 5). The leukocyte count increased by 53.90% in all treatments; similarly, erythrocytes increased by 53.30%.

Table 5. Hematologic parameters of the different treatments. Values represent mean values.

Initial	Control	Onion	Tequesquite	Ivermectin	SEM	P
% hematocrit	32.13	31.88	35.75	30.23	0.93	0.20
P.P. g/dL	7.97	6.91	7.25	7.31	0.14	0.06
Leukocytes 10^3/uL	17.33	14.88	16.83	17.76	0.97	0.75
Erythrocytes 10^6 /uL	1.38	1.41	0.92	1.04	0.08	0.08
Final						
% hematocrit	32.31	31.21	34.03	32.83	0.56	0.37
P.P. g/dL	7.72^{ab}	7.05^{b}	7.40^{ab}	8.30^{a}	0.17	0.05
Leukocytes 10^3/uL	41.63^{a}	22.42^{b}	28.05^{b}	30.81^{b}	1.99	0.002
Erythrocytes 10^6 /uL	4.09^{a}	3.61^{a}	2.88^{b}	2.18^{c}	0.15	0.0001

^{abc}Different letters within a row indicate significant difference ($P < 0.05$); P.P.=plasmatic proteins; SEM= standard error of mean.

4. Discussion

The deworming effect of onion may be due to the presence of bioactive agents such as saponins, sapogenins, flavonoids [15], terpenoids, and phenolic compounds [30]. Sulfur-rich compounds, such as thiosulfinates and polysulfides, have an effect on *Leshmania major, L. tropica, L. infantum L. mexmex L. donovani* [31], and *Schistosoma mansoni* [32]. The inclusion of onion in the diet may also have other beneficial effects; for example, there is evidence that onion extract increases (8.03%) total *in-vitro* gas production [30], which may be beneficial for sheep because of the icreased digestibility.

Due to the organic nature of tequesquite, it was not possible to determine its ruminal digestibility or mineral availability; however, it could modify the conditions in the rumen, specifically the presence of N-NH_3 [33]. The presence of other minerals in low concentrations, such as Zn and Cu, may have beneficial effects on the rumen microbiota [34]. In another study, Sr improved Ca absorption in ruminants [35], while Cr improved protein metabolism in lambs [36], immune response, and glucose turnover rate [37].

On the other hand, the trona present in the tequesquite possibly affects the integrity of the membrane through the peroxidation of polyunsaturated fatty acids in the organelles of hematophagous PGI cells, affecting the antioxidant capacity of the cell [38]. The presence of other elements, such as copper, can present multiple mechanisms such as protection against oxidative stress [39], which reduces the parasitic load. Therefore, due to its large number of compounds and elements, tequesquite may have a direct effect on the parasites, decreasing the load. Indirectly, it stimulates the response, improving the antioxidant capacity or nutritional condition of the ruminants.

The results of this study show that the most prevalent parasites were *Haemonchus* spp. (71.8%) and *Eimeria* spp. (71.8%); our values are higher than those reported for *Haemonchus* spp. in grazing systems in the states of Tabasco and Guerrero, Mexico, with 37% [11] and 32% [40], respectively. In another study conducted in Ontario, Canada, where measurements were performed at various periods of the year in grazing sheep, the authors found a prevalence of 50% of *Teladorsagia* spp. and 45% of *Haemonchus* spp.; however, during the months of July-August, the prevalence of *Teladorsagia* spp. decreased to 42%, while that of *Haemonchus* spp. remained the same (45%); at the beginning of autumn, the presence of these nematodes (*Haemonchus* spp. y *Teladorsagia* spp.) decreased to 26 and 36%, respectively [41]. In this study, the prevalence of *Eimeria* spp. was 71.8%, which was similar to that reported by Souza *et al*. [42], i.e. 80.2% in the rainy season and 55.8% in the dry season in grazing ruminants. The similar prevalences are a result of the fact that the sheep used in this experiment were obtained from a grazing system; although the climatic conditions are different from those reported, grazing is still a determining factor.

The productive parameters were not affected by the treatments. It should be mentioned that the diet provided was designed for maintenance, with the aim that the nutritional status had no effect on the parasite load, simulating the conditions of a grazing regime in the dry season, where the sheep would consume a diet with a minimum inclusion of a balanced feed [43]. On the other hand, no visible clinical changes were observed in the sheep; body weight did not significantly differ between the treatments, as reported by Ademola *et al*. [44], who applied an extract of *S. mombin* for the treatment of gastrointestinal parasites (*Haemonchus sp., Trichostrongylus spp. Oesophagostomum spp.*, *Strongyloides spp.*, and *Trichuris spp*.), without observing adverse clinical manifestations secondary to the application of the treatment; no changes were observed in the average values of final weight gain.

The use of anthelmintics applied in this experiment (onion, tequesquite, and ivermectin) did not induce any changes in the productive parameters and eggs per gram of feces over time (Table 3), suggesting that the treatment with ivermectin was not different from the natural treatments (onion and tequesquite), although we observed a decrease (≈ 50%) of the total parasite load, presumably due to the effect of anthelmintics used. We therefore infer that these natural treatments are effective against parasites; however, the underlying mechanisms need to be further explored.

The use of tequesquite significantly decreased the number of nematode eggs per gram of feces by 75.70% ($P = 0.03$), measured at the beginning and at end of the experiment (Table 4). These results differ from those reported by Schafer *et al*. [45], who evaluated the immune response, using Zn and Cu, with doses of 1.5 mg of Zn EDTA kg/LW, associated with 0.45 mg of Cu EDTA kg/LW, of the subcutaneously applied compound in sheep infected with *H. contortus*. The authors observed that the number of eggs per gram of feces and the adult prevalence in the sheep abomasum were not reduced. Possibly, the inclusion of tequesquite has a positive effect on the immune response of the host, which indirectly favors the reduction of the parasite load due to the mineral content [19].

The number of EPG decreased throughout the experiment in all treatments, although the parasite loads of *Haemonchus* spp., *Cooperia* spp., and *Strongyloides* spp. were maintained and even increased at the end of the sampling period in the group treated with ivermectin (Fig. 1). During the initial and final phases of the experiment (Table 5), the hematocrit values remained within the reference range of 24-35% [46], which can be explained by the fact that during the period of patency (presence of signs of disease and presence of eggs in feces), the hematocrit percentage decreases, with a subsequent recovery. This has been reported by Angulo *et al*. [47], who inoculated sheep with three isolates of *Haemonchus* spp., with the objective to determine the cellular response depending on the pathogenicity of the isolate, highlighting that the inoculation with *H. contortus* presents a reduction in the hematocrit of ovine animals, which possibly coincided with the onset of patency. McKinnon *et al*. [48] reported that there were no differences in the hematocrit (HTC) between days 16 and 21 of the sampling; however, on day 27, the hematocrit increased in sheep infected with *Haemonchus contortus,* which is similar to our results. This response can be associated with the stage of the life cycle in which the parasite is found, since *H. contortus* absorbs blood and causes hemorrhagic gastritis, followed by the recovery of the erythropoiesis. In addition, the treatment with onion did not have any negative effects, since in some species, it produces oxidative stress, which agrees with the findings of Kommuru *et al*. [49], who observed no effect on the HTC when using flour of *Lespedeza serices*. However, they found a decrease in the parasite load in goats. Plasma protein levels at the start of the experiment were within the reference range 6.1-7.2 g dL [46], although these values were increased in the control and in the treatment using ivermectin (Table 5). This can be explained by the fact that chronic nematode infections induce an immune response Th2, since during the first phase of the infection, there is an increase of globulins and a pro-inflammatory response, which leads to the increase of plasma proteins [50]. However, in the case of onion and tequesquite use, the plasma protein values were not modified, which is in agreement with the findings of Angulo *et al*. [47], who stated that the infection is accompanied by mild hypoproteinemia at the end of the pre-patent period. The leukocyte reference value is 7.8-12.7 x 103 uL [46], it can be observed that when comparing the initial and final samples, indicates that there was a positive cellular response against to the gastrointestinal nematodes. The control treatment presented the largest leukocyte count in comparison with the other treatments (Table 5), which corroborates the findings of Terefe *et al*. [51], who report that in the first days after inoculation with *H. contortus*, there is a slight increase in leukocytes, while in the second and third week, this increase is relevant for leukocytes and eosinophils. Similarly, MacKinnon *et al*. [48] observed that leukocytes increased from day 17 post-inoculation in a ratio of 4: 1 in relation to the control.

Throughout the experiments with onion and tequesquite, the erythrocyte value (Table 5) was lower than the reference value marked by Tschuor *et al*. [46], ranging from 13.5 to 18.4 x 10^6 uL. Values below this threshold lead us to infer that blood loss occurred, which can be related to the presence of gastrointestinal parasites, particularly *H. contortus*, a highly pathogenic organism and blood consumer [49].

5. Conclusion

Tequesquite decreased the number of eggs per gram of feces in a percentage similar to Ivermectin whereas onion treatment showed less effect in parasitic load than other treatments. The natural treatments had no adverse effects on the productive and hematological parameters of lambs fed with maintenance diet.

Natural treatments such as onion and tequesquite have two fundamental advantages over Ivermectin, first do not generate contamination of soil and water and second does not leave residues in meat and milk and therefore does not require withdrawal time.

Recomendations

Since a single method is not capable to diminishing parasitic loads in sheep in the long term it is important to consider that natural alternatives such those evaluated here, should be considered as part of an integral parasite control program which includes the implementation of rotational grazing, Famacha system evaluation, selection of resistant animals and biological control.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contributions

All authors contributed equally to this work.

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References

[1] L.S. Vieira, "Métodos alternativos de controle de nematóides gastrintestinais em caprinos e ovinos," *Revista Tecnologia y Ciência Agropecuaria*, vol. 2, no. 2, pp. 49–56, 2008.

[2] P.M. Hernandez, A.Z. Salem, M.M. Elghandour, M. Cipriano-Salazar, B. Cruz-Lagunas, L.M. Camacho, "Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* Lam. extracts in growing lambs," *Tropical Animal Health and Production*, vol. 46, no.1, pp. 173-178, 2014.

[3] R. Van den Brom, L. Moll, C. Kappert, P. Vellema, "*Haemonchus contortus* resistance to monepantel in sheep," *Veterinary Parasitology*, vol. 209, no. 3-4, pp. 278-280, 2015.

[4] L.A. Melville, D. McBean, A. Fyfe, S.J. Campbell, J. Palarea-Albaladejo, F. Kenyon, "Effect of anthelmintic treatment strategy on strongylid nematode species

composition in grazing lambs in Scotland," *Parasites & Vectors*, vol.9 no. 199, 2016. doi: 10.1186/s13071-016-1493-6

[5] M.F.J. Van Houtert, I.A. Barger, J.W. Steel, "Supplementary feeding and gastrointestinal nematode parasitism in young grazing sheep," *Proceedings of the New Zealand Society of Animal Production*, no. 56, pp. 94-98, 2006.

[6] J.B. Githiori, J. Hoglund, P.J. Waller and R.L. Baker, "The anthelmintic efficacy of the plant, *Albizia anthelmintica* against the nematode parasite *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice," *Veterinary Parasitology*, vol. 116, no. 1, pp. 23-34, 2003.

[7] F. Ramos, L.P. Pires, F.R. de Souza, C.R. Zamperete, L. Pötter, A.C. Skrebsky, L.A. Sangioni, F.S.V. Flores, "Anthelmintic resistance in gastrointestinal nematodes of beef cattle in the state of Rio Grande do Sul, Brazil," *International Journal for Parasitology: Drugs and Drug Resistance* vol. 6 no. 1, pp. 93-101, 2016.

[8] P.S. Nery, F.A. Nogueira, E.R. Martins, E.R. Duarte, "Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep," *Veterinary Parasitology*, vol. 171 no. 3-4, 361-364, 2010.

[9] J.T. Gradé, B.L. Arble, R.B. Weladji and P. Van Damme, "Anthelmintic efficacy and dose determination of *Albizia anthelmintica* against gastrointestinal nematodes in naturally infected Ugandan sheep," *Veterinary Parasitology*, vol. 157, no. 3-4, pp. 267-274, 2008.

[10] M. Adamu, O.D. Oshadu, C.I. Ogbaje, "Anthelminthic efficacy of aqueous extract of *Acanthus montanus* leaf against strongylid nematodes of small ruminants," *African Journal of Traditional, Complementary and Alternative medicines*, vol. 7, no. 4, pp. 279-285, 2010.

[11] G.R. González, P.C. Córdova, H.G. Torres, G.P. Mendoza, G.J. Arece, "Prevalence of gastrointestinal parasites in slaughtered sheep at a slaughterhouse in Tabasco, Mexico," *Veterinaria México*, vol. 42 no. 2, pp. 125-135, 2011.

[12] B.R. Joshi, D.S. Kommuru, T.H. Terrill, J.A. Mosjidis, J.M. Burke, K.P. Shakya, J.E. Miller, "Effect of feeding *Sericea lespedeza* leaf meal in goats experimentally infected with *Haemonchus contortus," Veterinary Parasitology*, vol. 178, no. 1-2, 192–197, 2011.

[13] S.G. Lopes, L.B.G. Barros, H. Louvandini, A.L. Abdalla, L.J. Costa, "Effect of tanniniferous food from *Bauhinia pulchella* on pasture contamination with gastrointestinal nematodes from goats," *Parasites & Vectors,* vol. 9 no.102, 2016. DOI 10.1186/s13071-016-1370-3

[14] M. de Aquino Mesquita, J.B. E Silva Júnior, A.M. Panasso, E.F. de Oliveira, A.L. Vasconcelos, H.C. de Paula, C.M. Bevilaqua, "Anthelmintic activity of *Eucalyptus staigeriana* encapsulated oil on sheep gastrointestinal nematodes," *Parasitology Research*, no. 112 no. 9, pp. 3161–3165, 2013.

[15] V. Lanzotti, "The analysis of onion and garlic" *Journal of Chromatography A*, vol. 1112 no. 1-2, pp. 3–22, 2006.

[16] J.B. Githiori, S. Athanasiadou and S.M. Thamsborg, "Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants," *Veterinary Parasitology*, vol. 139, no. 4, pp. 308-320, 2006.

[17] E. Block, "The chemistry of garlic and onions," *Scientific American,* vol. 252, no. 3 pp. 114-119, 1985.

[18] A.A. Salama, M. AbouLaila, M.A. Terkawi, A. Mousa, A. El-Sify, M. Allaam, A. Zaghawa, N. Yokoyama, I. Igarashi, "Inhibitory effect of allicin on the growth of

Babesia and *Theileria equi* parasites," *Parasitology Research*, vol. 113, no. 1, pp. 275-283, 2014.

[19] S. Bhattacharjee, A. Sultana, M. Hasnan, M. Ariful, M.M. Ahtashom, Asaduzzaman, "Analysis of the proximate composition and energy values of two varieties of onion (*Allium cepa* L.) bulbs of different origin: A comparative study," *International Journal of Nutrition and Food Sciences,* vol. 2, no. 5, pp. 246-253, 2013.

[20] A. Vidyashankar, B. Hanlon and R. Kaplan, "Statistical and biological considerations in evaluating drug efficacy in equine strongyle parasites using fecal egg count data," *Veterinary Parasitology*, vol. 185, no. 1, pp. 45-56, 2012.

[21] AOAC. *Official methods of analysis*, 18th edn. Association of Official Analytical Chemists, Washington, DC, USA, 2005.

[22] P.J. Van Soest, J.B. Robertson and B.A. Lewis, "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition," *Journal of Dairy Science*, vol. 74, no. 10, pp. 3583-3592, 1991.

[23] E.S. Vanzant, R.C. Cochran and E.C. Titgemeyer, "Standardization of *in situ* techniques for ruminant feedstuff evaluation," *Journal of Animal Science*, vol. 76, no. 10, pp. 2717- 2729, 1998.

[24] G.V. Pashkova, A.N. Smagunova, A.L. Finkelstein, "Possibilities of the X-ray fluorescence analysis of dairy products with the help of the spectrometer with total external reflectance," *Chemistry for Sustainable Development*. Vol. 19, no. 2011, pp. 271-280, 2011.

[25] J.M. Forbes. “Introduction. Methods of measuring food intake,” In: Forbes JM (ed). Voluntary food intake and diet selection in farm animals. 2nd ed. Biddles Ltd, King’s Lynn, London, UK. pp 1-40, 2007.

[26] I.B. Wood, N.K. Amaral, K. Bairden, J.L. Duncan, T. Kassai, J. B.Jr. Malone, J.A. Pankavich, R.K. Reinecke, O. Slocombe, S.M. Taylor and J. Vercruysse, “World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine),” *Veterinary Parasitology,* vol. 58, no. 3, pp. 181-213, 1995.

[27] N.C. Jain, Schalm’s Veterinary hematology, 4th edn. Lea and Febiger, Philadelphia, pp. 20–80, 1986.

[28] J. Sall, A. Lehman, M. Stephens, L. Creighton, “JMP® Start Statistics: A Guide to Statistics and Data Analysis,” 5th edn. (SAS Institute Inc: Cary, NC, USA), 2012.

[29] G.D.R. Steel, J.H. Torrie and D.A. Dickey, 1997. Principles and procedures of statistics: a biometrical approach, 3rd edn. McGraw-Hill, New York, NY.

[30] E.T. Kim, C.H. Kim, K.S. Min, S.S. Lee. “Effects of Plant Extracts on Microbial Population, Methane Emission and Ruminal Fermentation Characteristics in *In vitro*,” *Asian-Australasian Journal of Animal Science*, vol. 25, no. 6, pp. 806 – 811, 2012.

[31] S. Danish Saleheen, M. Atif Ali, Masoom Yasinzai, “Antileishmanial activity of aqueous onion extract *in vitro*,” *Fitoterapia,* vol. 75, no. 2004, pp. 9–13, 2004.

[32] M.M.1.Mantawy, H.F. Aly, N. Zayed, Z.H. Fahmy. “Antioxidant and schistosomicidal effect of *Allium sativum* and *Allium cepa* against *Schistosoma*

mansoni different stages," *European Review for Medical and Pharmacological Science*, no.16, vol. 3, pp. 69-80, 2012.

[33] J.D. Urías-Estrada, M.A. López-Soto, A. Barreras, J.A. Aguilar-Hernández, V.M. González-Vizcarra, A. Estrada-Angulo, R.A. Zinn, G.D. Mendoza and A. Plascencia, "Influence of zeolite (clinoptilolite) supplementation on characteristics of digestion and ruminal fermentation of steers fed a steam-flaked corn-based finishing diet, *Animal Production Science*," 2017. http://dx.doi.org/10.1071/AN16128

[34] J.F. Vázquez-Armijo J.J. Martínez-Tinajero, D. López, Z. Abdel-Fattah, M. Salem, R. Rojo, "*In vitro* gas production and dry matter degradability of diets consumed by goats with or without copper and zinc supplementation," *Biological Trace Element Research,* no. 144, pp. 580–587, 2011. DOI 10.1007/s12011-011-9113-y

[35] M.L. Hyde and D.R. Fraser, "*In vivo* measurement of the absorption of strontium in the rumen and small intestine of sheep as an index of calcium absorption capacity," *British Journal of Nutrition*, no. 112, pp. 718–724, 2014.

[36] V.I.A. Domínguez, S.S. González, J.M. Pinos, J.L. Bórquez, R. Bárcena, G. Mendoza, L.E. Zapata, L.L. Landois, "Effects of feeding selenium-yeast and chromium-yeast to finishing lambs on growth, carcass characteristics, and blood hormones and metabolites," *Animal Feed Science and Technology*, vol. 52, pp. 42–49, 2009.

[37] J.W. Spears, and W.P. Weiss, "Mineral and vitamin nutrition in ruminants," *The Professional Animal Scientist*, vol. 30, pp. 180–191, 2012.

[38] T.O. Ajiboye1, Y.O. Komolafe, M.T. Yakubu, S.M. Ogunbode, “Effects of trona on the redox status of cellular system of male rats,” *Toxicology and Industrial Health,* vol. 31, no. 2, pp. 179–187, 2015.

[39] A.F.Vatta, P.J. Wallerb, J.B. Githiori, G.F. Medley, “Persistence of the efficacy of copper oxide wire particles against *Haemonchus contortus* in grazing South African goats,” *Veterinary Parasitology,* vol. 190, no. 1–2, 23, pp. 159-166, 2012.

[40] S. Rojas-Hernández, I. Gutiérrez-Segura, J. Olivares-Pérez, M.T. Valencia-Almazán, “Prevalencia de nemátodos gastrointestinales en ovinos en pastoreo en la parte alta del MPIO. De Cuetzala del Progreso, Guerrero-México,” *REDVET. Revista electrónica de Veterinaria*. Vol. 8, no. 9, pp. 1-7, 2007.

[41] A. Mederos, S. Fernández, J. VanLeeuwen, A.S. Peregrine, D. Kelton, P. Menzies, A. LeBoeuf, R. Martin, “Prevalence and distribution of gastrointestinal nematodes on 32 organic and conventional commercial sheep farms in Ontario and Quebec, Canada (2006-2008),”. *Veterinary Parasitology*, vol. 170, no. 3-4, pp. 244-52, 2010.

[42] L.E. de Souza, J.F. da Cruz, M.R. Teixeira-Neto, G.R. Albuquerque, A.D. Melo, D.M. Tapia, “Epidemiology of *Eimeria* infections in sheep raised extensively in a semiarid region of Brazil,” *The Brazilian Journal of Veterinary Parasitology*, vol. 24, no. 4, pp. 410-415, 2015.

[43] P. Vázquez-Mendoza, O.A. Castelán-Arteaga, A. García-Martínez and F. Avilés-Nova, “Uso de bloques nutricionales como complemento para ovinos en el trópico seco del altiplano central de México,” *Tropical and Subtropical Agroecosystems*, vol. 15, 87-96, 2012.

[44] I.O. Ademola, B.O. Fagbemi, S.O. Idowu, “Anthelmintic activity of extract of *Spondias mombin* against gastrointestinal nematodes of sheep: Studies *In vitro* and *In vivo,” Tropical Animal Health and Producction*, vol. 37, pp. 223-235, 2005.

[45] A.S. Schafer, M.L.R. Leal, M.B. Molento, A.R. Aires, M.M.M.F. Duarte, F.B. Carvalho, A.A. Tonin, L. Schmidt, E.M.M. Flores, R.T. Franca, T.H. Grando, A.P. Minho, A. Krause, A.Q. Antoniazzi, S.T.A. Lopes, “Immune response of lambs experimentally infected with *Haemonchus contortus* and parenterally treated with a combination of zinc and copper,” *Small Ruminant Research*. Vol. 123 no.1, pp. 183-188, 2015.

[46] A.C. Tschuor, B. Riond, U. Braun, H. Lutz, “Hämatologische und klinisch-chemische Referenzwerte für adulte Ziegen und Schafe” *Schweizer Archiv Fur Tierheilkunde*, vol. 150, no. 6, pp. 287–295. 2008.

[47] F.J. Angulo, L. García, J.M. Alunda, M. Cuquerella, C. de la Fuente, “Biological characterization and pathogenicity of three *Haemonchus contortus* isolates in primary infections in lambs,” *Veterinary Parasitology*, vol. 171, no. 2010, pp. 99–105, 2010.

[48] K.M. MacKinnon, A.M. Zajac, F.N.J. Kooyman, D.R. Notter, “Differences in immune parameters are associated with resistance to *Haemonchus contortus* in Caribbean hair sheep,” *Parasite Immunology*, vol. 32 no. 7(2010), pp. 484–493, 2010.

[49] D.S. Kommuru, N.C. Whitley, J.E. Miller, J.A. Mosjidis, J.M. Burke, S., Gujja, A. Mechineni, T.H. Terrill, “Effect of *Sericea lespedeza* leaf meal pellets on adult female *Haemonchus contortus* in goats” *Veterinary Parasitology*, vol. 207, no. 1-2, pp. 170-175, 2015.

[50] N.M. Resende, P.H. Gazzinelli-Guimarães, F.S. Barbosa, L.M. Oliveira, D.S. Nogueira, A.C. Gazzinelli-Guimarães, M.T. Gonçalves, C.C. Amorim, Oliveira F.M. Caliari, M.V. M.A. Rachid, G.T. Volpato, L.L. Bueno, S.M. Geiger, R.T. Fujiwara, "New insights into the immunopathology of early *Toxocara canis* infection in mice," *Parasites & Vectors*, vol. 8, no. 354, 2015. doi: 10.1186/s13071-015-0962-7

[51] G. Terefe, C. Lacroux, O. Andreoletti, C. Grisez, F. Prevot, J.P. Bergeaud, J. Penicaud, V. Rouillon, I. Gruner, J.C. Brunel, D. Francois, J. Bouix, P. Dorchies and P. Jacquiet, "Immune response to *Haemonchus contortus* infection in susceptible (INRA 401) and resistant (Barbados Black Belly) breeds of lambs," *Parasite Immunology*, vol. 29, no. 8, pp. 415-424, 2007.

8.3. Artículo 3

Genetic diversity of *Haemonchus contortus* identified from grazing sheep in the State of Mexico

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Abstract

Ovines in extensive production are frequently affected by gastrointestinal nematodes, which cause great economic losses, *Haemonchus contortus* is a hematophagous nematode with worldwide distribution, it has been reported in several studies that has high resistance to anthelmintics, its identification by microscopy is complicated since the eggs are very similar to those of other strongyles, so molecular diagnosis is the tool of choice to know in detail the species genus even if it is a resistant isolate. For this study, 24 ovines naturally infected with gastrointestinal parasites were used, samples were taken directly from the rectum and analyzed by microscopy and later by PCR, the ITS-2 rDNA region was amplified, positive samples were sequenced and aligned with samples reported worldwide. Fragments of 315 base pairs of 16 samples were amplified which were identified as *Haemonchus contortus* and presented identity of 99% to 1oo% with isolates reported in; Laos, New Zealand, Thailand, Brazil and USA. The identification of *Haemonchus contortus* in sheep from the southeastern part of the State of Mexico is of great importance because genetic diversity and identity were found with isolates from other countries. The results suggest that, in addition to anthelmintic resistance, it has climatic adaptability.

Key words: molecular diagnosis, anthelmintic resistance, grazing, sheep, *Haemonchus contortus*

Introduction

Small ruminants are an important source of protein for human consumption (Warleed *et al*., 2017), One of the main problems affecting sheep in production is the high prevalence of gastrointestinal nematodes (Rowe *et al*., 2008) the most important worldwide is *Hemonchus contortus* (Moura *et al*., 2016), clinical signs in infected animals include hyporexia or anorexia and modification in the digestibility of food, due to damage caused to the light of the digestive tract, these alterations are reflected in the decrease of production, the quality of milk, meat and the reproductive efficiency of the host (Sato *et al*., 2014).

Recent advances in molecular biology have provided means to identify nematode eggs and / or larval stages with much greater reliability than microscopic techniques, the first and the second internal transcribed space (ITS-1 e ITS-2) of ADN ribosomal (ADNr) have proved to be particularly useful sources of specific markers of species and / or genus for this purpose (Bisset *et al*., 2014), Therefore, the objective of this study was to identify *Haemonchus contortus* by PCR, stool samples of naturally infected sheep as well as to determine if there is genetic diversity in the samples obtained in the southeastern zone of the State of Mexico.

Materials and Methods

Animals

Twenty-four naturally-occurring criollo sheep grazing naturally growing in the south-eastern part of the State of Mexico, weighing 22 kg +/- 2 kg, were randomly selected, stablished in individual cages in the zootechnical post of the UAEM Amecameca

University Center and 60% corn stubble and 40% commercial concentrate were provided with maintenance diet. The experiment was reviewed and approved by the Ethics and Animal Welfare Committee of the UAEM Amecameca University Center of the Autonomous University of the State of Mexico.

Samples

The sheep were sampled during the stable and once a week for four weeks, direct rectal stool was obtained by digital stimulation, the samples were collected in polyethylene bags and analyzed immediately in the Parasitology laboratory of the Animal Veterinary Clinic. CLIVAC of the UAEM Amecameca University Center, the Faust technique was used for the morphometric identification of the parasites present and the McMaster technique was used to determine the parasitic load expressed in eggs per gram of feces (EPG), the samples positive for *Haemonchus* spp. They were taken to the Biotechnology laboratory of the UAEM Amecameca University Center for analysis.

DNA extraction and sequencing

DNA extraction was done directly from the stool with the commercial kit Extraction DNA ZR Fecal DNA miniprep Zymo Reseach following the manufacturer's instructions, The ITS-2 rDNA region was amplified, using the primers NC1F (5-ACGTCTGGTTCAGGGTTGTT-3) and NC2R (5 TTAGTTTCTTTTCCTCCGCT-3) (Stevenson *et al*., 1995; Brasil *et al*.., 2012). The PCR was performed at a volume of 25 µL, with 8.75 µL H2O nuclease-free, 5 µL of Green, 3.0 µL of MgCl2, 1 µL dNTP, 1 µL of each primer, 0.25 µL of GoTaq DNA polymerase (Promega) and 5 µL of the DNA obtained. The conditions for the reaction were given by an automatic thermal cycler (marca del termociclador) with the following protocol, initial denaturation to 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 1 minute, with final extension at 72°C for 10 minutes. The PCR products (5µ) were visualized on 2% agarose gels and selected for direct sequencing. The sequences were performed in MacroGen, Rockville, USA.

Analysis of data

The sequences were aligned with the MEGA 6 program for the multiple alignment of sequences for the evidence of recombination by examining each possible sequence triplet and a dendrogram of association was made with the sequences obtained and those reported worldwide.

Results

fragments of 315 bp were amplified as shown in Figure 1, 16 seqeuences belonging to the genus *Haemonchus* and were identified to species level based on region sequences of ITS-2 rDNA, the sequences found presented identity of 99% to 1% with three different alleles of isolates reported in; Laos (AB908961.1, AB908963.1, AB908962.1), with two of New Zealand (KC998714.1, KC998713.1), three of Thailand (KP101382.1, KP101380.1, KP101379.1), six of Brazil (JN128897.1, JN128898.1, JQ342246.1, JQ342247.1, JN128898, JQ342248) and 18 of USA (EU086393.1, EU086378.1, EU084691.1, EU084689.1, EU084687.1, EU084684.1, EU086382.1, EU086383.1, EU086381.1, EU084688.1, EU084686.1, EU084683.1, EU086387.1, EU086385.1, EU086390.1, EU086389.1, EU086386.1, EU086384.1) These reference sequences of *Haemonchus contortus* are deposited in the GenBank, with which the association dendogram was made, which is shown in Figure 2, where the sequences of the isolates of New Zealand, Laos, USA with a sequence from Mexico are grouped and in another clade the sequences of the isolates are grouped from Laos, New Zealand, Thailand, Brazil, USA and the rest of the sequences from Mexico.

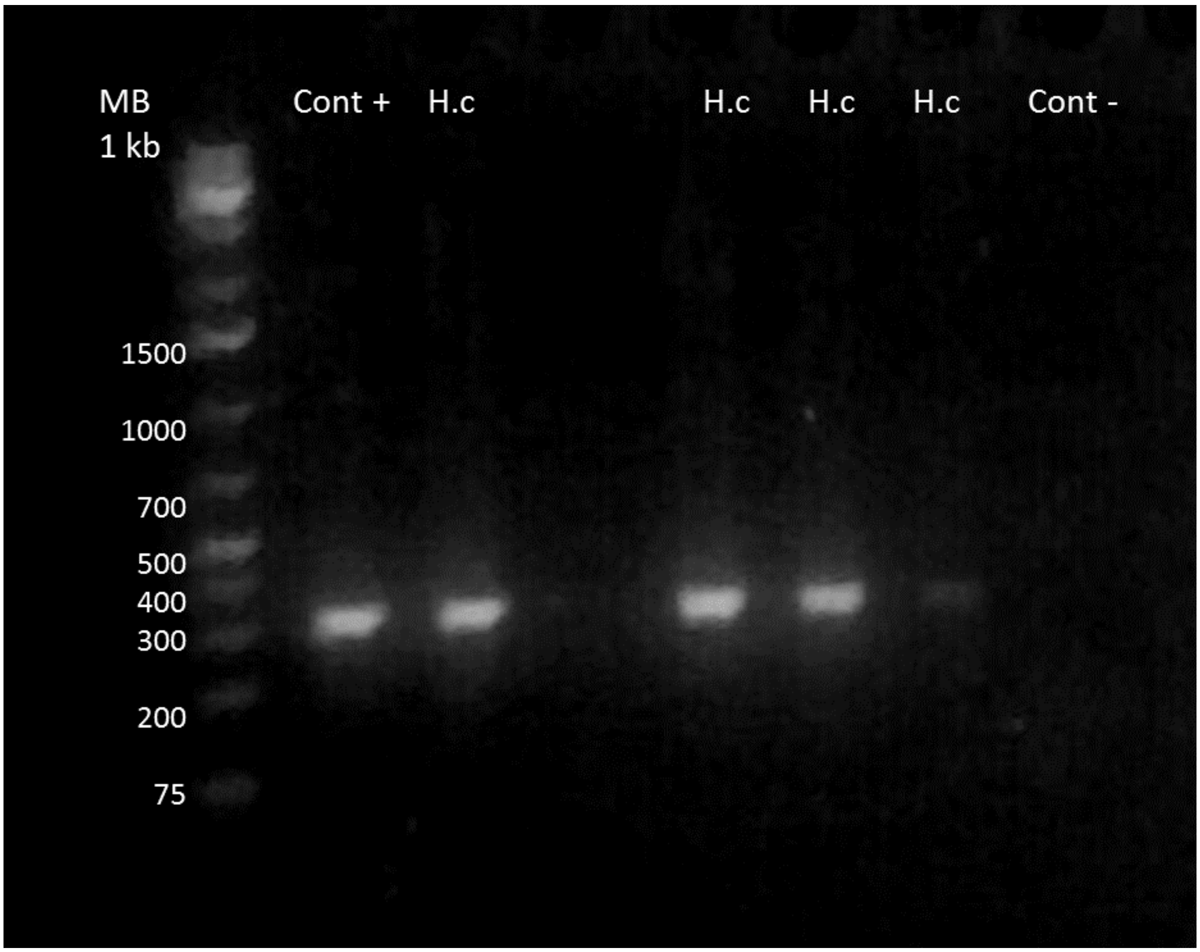

Fig. 1. 2% agarose gel showing the amplification of 315 bp fragments corresponding to the ITS2 region of *H. contortus*, obtained in stool samples of sheep from Mexico. MB; Molecular weight marker, 1-6) Positive samples to *H. contortus*, 7) negative control (sheep DNA).

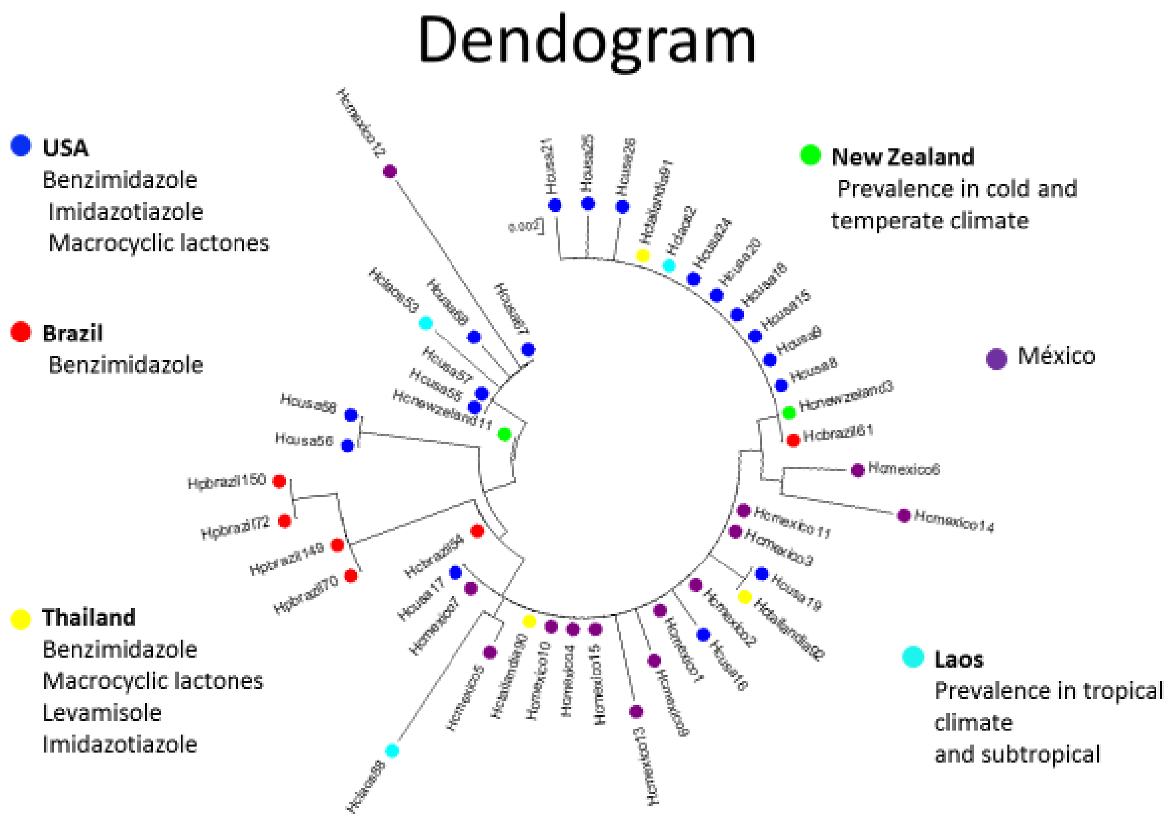

Fig. 2. Dendogram of association of the sequences obtained with those reported.

Discussion

Molecular biology techniques are effective in taxonomy, especially in cases where morphological analysis can be confusing, as in the identification of *Haemonchus* spp. (Brasil *et al*., 2012) since the eggs eliminated in the feces are morphometrically similar to *Ostertagia* spp. *Trichostrongylus* spp. and others Trichostrongyles, so the PCR technique is the ideal (Bisset *et al*., 2014) as it happened in this study since of the 24 stool samples from sheep positive to *Haemonchus* spp. by microscopy, 16 were positive for *Haemonchus contortus* by PCR, it is said that 100% of the samples presented eggs of Trichostrongyle (*Haemochus* spp. *Ostertagia* spp. *Trichostrongylus* spp.) by microscopy and of these 66.6% were positive for *H. contortus* by PCR. The genetic structure of *Haemonchus* populations is generally low within contiguous geographic regions as countries, but is high significantly globally (Brasil *et al*., 2012). In the dendogram we can see how the sequences are divided into two clades, in the first we group sequences reported in the USA that show resistance to the benzimidazole, imidazotiazole and macrocyclic lactones (Garretson *et al*., 2009) and with a sequence reported in Laos that presents high prevalence in tropical and subtropical climate (Sato *et al*., 2014), in the other clade there are sequences reported in New Zealand with prevalence in cold climate as temperate (Bisset *et al*., 2014), USA with resistance to benzimidazole, imidazothiazole and macrocyclic lactones (Garretson *et al*., 2009), Brazil with resistance to benzimidazole (Brasil *et al*., 2012), Thailand resistance to benzimidazole, macrocyclic lactones, levamisole and imidazothiazole (Mangkit *et al*., 2014) and the other sequences of the State of Mexico, this could be a consequence of the high gene flow and the low genetic structure that is frequently found in trichostrongylid populations, which is caused by the frequent movement of animals between farms in different states and even between countries, on the other hand the combination of different species of ruminants in grazing areas or facilities (Brasil *et al*., 2012; Nunes *et al*., 2013), This is a common situation in Mexico, since the regulations allow the movement of animals throughout the national territory without a certificate confirming that the animals are free of endoparasites.

Trichostrongylids present high prevalences in tropical and temperate climates (Brasil *et al*., 2012) however, the climate of the south east zone of the state of Mexico has a temperate to cold climate, which due to the characteristics of the samples analyzed in this study suggest that they come from *Haemonchus contortus* resistant to anthelmintics and with adaptation to cold climates since part of their cycle they pass on the ground exposed to the environment.

Conclusions

It was identified by PCR *Haemonchus contortus* in stool samples from sheep from the southeastern area of the State of Mexico, genetic diversity and identity were found with isolates that show resistance to anthelminthic treatments in other countries, and by the climatic characteristics of the area where obtained samples of *Haemonchus contortus* for this study, the results suggest that, in addition to anthelminthic resistance, it also shows resistance to cold climates. Molecular diagnosis has practical advantages, since it can guide the choice of the anthelmintic drug to be used, before its application in the herd, thus reducing the economic losses caused by anthelmintic resistance.

Conflict of interest

The authors have no conflict of interest.

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References

Arafa W.M., Holman P.J., Craig T.M. Genotypic and phenotypic evaluation for benzimidazole resistance or susceptibility in *Haemonchus contortus* isolates. Parasitology Research. 2017;116(2):797-807.

Bisset S.A., Knight J.S., Bouchet C.L.G. A multiplex PCR-based method to identify strongylid parasite larvae recovered from ovine faecal cultures and/or pasture samples. Veterinary Parasitology. 2014; 200(2014):117–127.

Brasil S.A.F.B., Nunes R.L., Bastianetto E., Drummond M.G., Carvalho D.C., Leite R.C., Molento M.B., Oliveira D.A.A. Genetic diversity patterns of *Haemonchus placei* and *Haemonchus contortus* populations isolated from domestic ruminants in Brazil. International Journal for Parasitology. 2012; 42(2012):469–479.

Garretson P.D., Hammond E.E., Craig T.M., Holman P.J. Anthelmintic resistant *Haemonchus contortus* in a giraffe (*Giraffa camelopardalis*) in Florida. Journal of Zoo and Wildlife Medicine. 2009; 40(1):131–139.

Mangkit B., Thaenkham U., Adisakwattana P., Watthanakulpanich D., Jantasuriyarat C. and Komalamisra C. Molecular Characterization of *Haemonchus contortus* (Nematoda: Trichostrongylidae) from Small Ruminants in Thailand Based on the Second Internal Transcribed Spacer of Ribosomal DNA. Kasetsart Journal (Nat. Sci.). 2014; 48:740-758.

Moura C.A., Dantas S.F.J., Pacheco A., Batista da Cunha A., dos Santos Cruz J., Scofield A., Góes-Cavalcante G. F200Y polymorphism of the β-tubulin isotype 1 gene in *Haemonchus contortus* and sheep flock management practices related to anthelmintic resistance in eastern Amazon. Veterinary Parasitology. 2016;226(2016):104–108.

Nunes R.L., dos Santos L.L., Bastianetto E., Andrade O., Aparecida D., Brasil S.A.F.B. Frequency of benzimidazole resistance in *Haemonchus contortus* populations isolated from buffalo, goat and sheep herds. Revista Brasileña de Parasitololgía Veterinaria Jaboticabal. 2013; 4(22):548-553.

Rowe A., Yun K., Emery D., Sangster N. *Haemonchus contortus*: Development of a two-step, differential-display PCR to detect differential gene expression in nematodes from immune and naı¨ve sheep. Experimental Parasitology. 2008; 119(2008):207–216.

Stevenson L.A., Chilton N.B., Gasser R.B. Differentiation of *Haemonchus placei* from *H. contortus* (Nematoda: Trichostrongylidae) by the ribosomal DNA second internal transcribed spacer. International Journal of Parasitology. 1995; 25:483–488.

Sato M.O., Sato M., Chaisiri K., Maipanich W., Yoonuan T., Sanguankiat S., Pongvongsa T., Boupha B., Moji K., Waikagul J. Nematode infection among ruminants in monsoon climate (Ban-Lahanam, Lao PDR) and its role as food-borne zoonosis. Brazilian Journal of Veterinary Parasitology Jaboticabal. 2014; 1(23):80-84.

8. DISCUSIÓN GENERAL

Los resultados de este estudio muestran que los parásitos con más prevalencia fueron *Haemonchus* spp. 71.8%, y *Eimeria* spp., 71.8%, lo cual es mayor a lo reportado para *Haemonchus* spp., en sistemas de pastoreo en los estados de Tabasco, y Guerrero, México, con un 37 % (González *et al*., 2011) y 32 % (Rojas *et al*., 2007) respectivamente. En otro estudio desarrollado en Ontario, Canada; donde realizaron mediciones en varios periodos del año, en ovinos en pastoreo, reportan prevalencia de 50% de *Teladorsagia* spp. y el 45 % de *Haemonchus* spp., sin embargo, existieron cambios en la prevalencia durante los meses de julio–agosto donde *Teladorsagia* spp. disminuyó a 42%, *Haemonchus* spp. permaneció igual (45%), al inicio del otoño disminuye la presencia estos nematodos (*Haemonchus* spp. y *Teladorsagia* spp.) a 26% y 36%, respectivamente (Mederos *et al*., 2010). La prevalencia de *Eimeria* spp. observada en este estudio fue de 71.8 %, la cual fue similar a la reportada por Souza *et al*. (2015) en época de lluvia 80.2 % y en secas del 55.8 % en rumiantes en pastoreo, las prevalencias son similares debido a que los ovinos de este experimento provenían de sistema de pastoreo y aunque las condiciones del clima son diferentes con las reportadas, el pastoreo sigue siendo un factor determinante.

Los parámetros productivos no fueron afectados, cabe mencionar que la dieta proporcionada fue diseñada para el mantenimiento, con el objetivo de que el estado nutricional no tuviera efecto sobre la carga parasitaria, simulando que los ovinos se encontraban en un régimen de pastoreo en época de estiaje consumiendo esquilmos agrícolas y con una inclusión mínima de alimento balanceado. Por otra parte, no se observaron cambios clínicos visibles en los ovinos y los pesos no tuvieron diferencia significativa entre tratamientos como reportan Ademola *et al*. (2005) donde aplicaron un extracto de *S. mombin* para el tratamiento de parásitos gastrointestinales (*Haemonchus* sp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Trichuris* spp.) sin observar manifestaciones clínicas adversas secundarias a la aplicación del tratamiento y no se observaron cambios en la media de ganancia de peso final.

El uso de los antihelmínticos empleados en este experimento (cebolla e ivermectina) no presentaron diferencias significativas a través del estudio, incluso el tratamiento con ivermectina no fue diferente al tratamiento con *Allium cepa*, aunque se puede observan una tendencia en la disminución (≈ 50 %) de la carga parasitaria total con el uso de esta, el efecto antiparasitario de la cebolla puede deberse a la presencia de agentes bioactivos como saponinas, sapogeninas, flavonoides (Lanzotti, 2006), terpenoides y compuestos fenólicos (Kim *et al*., 2012), compuestos ricos en azufre tales como tiosulfinato, polisulfuros, se informa que tienen un efecto sobre *Leshmania major*, *L. tropica*, *L. infantum L. mexmex L. donovani* (Salesheen *et al*., 2004) y *Schistosoma mansoni* (Mantawy *et al*., 2012). Su inclusión en la dieta puede tener otros efectos beneficiosos, existen pruebas en las evaluaciones de la producción de gases *in vitro* donde el uso de extractos de cebolla aumento (8.03%) la producción total de gas (Kim *et al*., 2012), que puede ser beneficioso para las ovejas ya que aumentaría la digestibilidad, se podría sugerir que si tiene un efecto de disminución del número de huevos por gramo de heces.

El número de HPG disminuyo a lo largo del experimento en todos los tratamientos, aunque la carga parasitaria de *Haemonchus* spp., *Cooperia* spp. y *Strongyloides* spp., se mantuvieron e incluso aumentaron al finalizar los muestreos en el grupo tratado con ivermectina.

Durante la fase inicial y final del experimento, los valores de hematocrito se comportaron dentro del rango de referencia 24 - 35 % (Tschuor *et al*., 2008), lo cual se puede explicar pues durante el periodo de patencia (presencia de signos de enfermedad y presencia de huevos en heces), existe una disminución del porcentaje de hematocrito, posteriormente se recupera y se mantiene estable, tal como lo reportado por Angulo *et al*. (2010) quienes inocularon en ovinos con tres aislados de *Haemonchus* spp., con el objetivo determinar la respuesta celular dependiendo de la especie, destacando que *H. contortus* presenta una reducción en los hematocritos, la cual posiblemente fue coincidente con el inicio de la patencia. Por su parte McKinnon *et al*. (2010) reportan que no existieron diferencias en los hematocritos (HTC), a los días 16 y 21 de muestreo, sin embargo en el día 27 incrementó este parámetro inmunológico en ovejas infectadas con *Haemonchus contortus*, similar a lo encontrado en los resultados de este trabajo, con el uso de *Allium cepa* (cebolla), esta respuesta se puede asociar con la etapa del ciclo de vida en que se encuentre el parásito, ya que *H. contortus,* absorbe sangre y provoca gastritis hemorrágica, esto seguido de la recuperación de la eritropoyesis. Además, se puede considerar que el tratamiento con la cebolla en donde no se observó un efecto negativo en el hematocrito, ya que posiblemente puede provocar estrés oxidativo en algunas especies de parásitos, lo cual concuerda con lo reportado por Kommuru *et al*. (2015) donde no encontraron efecto sobre los HTC al emplear harina de hojas de *Lespedeza serices*, pero encontrando una disminución en la carga parasitaria en cabras.

Las proteínas plasmáticas al inicio estuvieron dentro del rango de referencia 6.1 - 7.2 g dL (Tschuor *et al*., 2008) aunque estos valores se incrementaron en el caso del tratamiento testigo y el uso de la ivermectina, esto se debe a que las infecciones crónicas de nematodos inducen una respuesta inmune Th2, ya que, durante la primera fase de la infección, hay aumento de globulinas y una respuesta pro-inflamatoria lo que conlleva el aumento de proteínas plasmáticas (Resende *et al*., 2015). En el caso del uso de cebolla no se modificaron los valores de proteínas plasmáticas, situación que fue similar a lo reportado por Angulo *et al*. (2010) mencionando que la infección se acompaña con ligera hipoproteinemia observándose en el final del periodo pre-patente.

En el caso del aumento en los leucocitos se cuenta como referencia con los siguientes valores de 7.8 -12.7 x 103 uL (Tschuor *et al*., 2008); al comparar los muestreos inicial y final, indica que existió un respuesta celular positiva a los nematodos gastrointestinales, el tratamiento testigo presento la mayor cuenta de leucocitos en comparación con los otros tratamientos, lo concuerda con Terefe *et al*. (2007) quienes comunican que en los primeros días post-inoculación con *H. contortus* existe un incremento menor en leucocitos, pero en las segunda y tercer semana de incubación este incremento se muestra relevante para leucocitos y eosinofilos. Caso contrario con lo encontrado por MacKinnon *et al*. (2010) puesto que los leucocitos se incrementaron a partir del día 17 post-inoculación en una proporción 4:1 en relación a su grupo testigo.

Durante toda la prueba con el uso de cebolla el valor de eritocitos, fue menor a la referencia marcado por Tschuor *et al*. 2008, que va de 13.5 a 18.4 x 106 uL, los valores inferiores a estos nos hacen inferir que existió pérdida de sangre, y podemos correlacionar con la presencia de parásitos gastrointestinales, particularmente *H. contortus*, organismo altamente patógeno y consumidor de sangre (Kommuru *et al*., 2015).

La estructura genética de las poblaciones de *Haemonchus* es por lo general baja dentro de regiones geográficas contiguas como países, pero significativamente alta a nivel mundial (Brasil *et al*., 2012). En el dendográma se puede observar cómo se dividen las secuencias en dos clados, en el primero se agrupan secuencias reportadas en USA que presentan resistencia a los benzimidazoles, imidazotiazoles y lactonas macrocíclicas (Garretson *et al*., 2009) y con una secuencia reportada en Laos que presenta alta prevalencia en clima tropical y subtropical (Sato *et al*., 2014), en el otro clado se encuentran secuencias reportadas en Nueva Zelanda con prevalencia en clima frio como templado (Bisset *et al*., 2014), USA con resistencia a benzimidazoles, imidazotiazoles y lactonas macrocíclicas (Garretson *et al*., 2009), Brasil con resistencia a benzimidazoles (Brasil *et al*., 2012), Tailandia resistencia a benzimidazoles, lactonas macrocíclicas, levamisol e imidazotiazoles (Mangkit *et al*., 2014) y las demás secuencias del Estado de México, esto podría ser una consecuencia del alto flujo de genes y la baja estructura genética que se encuentra comúnmente en poblaciones de trichostrongylidos, que es causada por la constante movilización de animales entre granjas de diferentes estados e incluso entre países, por otro lado la combinación de diferentes especies de rumiantes en áreas de pastoreo o instalaciones (Brasil *et al*., 2012; Nunes *et al*., 2013), situación común en México, ya que la normatividad permite la movilización de animales a través del territorio nacional sin un certificado que asegure que los animales están libres de endoparásitos. Los trichostrongylidos es de altas prevalencias en climas tropicales y templados (Brasil *et al*., 2012), sin embargo el clima de la zona sur oriente del estado de México tiene un clima de templado a frio, que por las características de las muestras analizadas en este estudio sugieren que provienen de *Haemonchus contortus* resistentes a antihelmínticos y con adaptación a climas fríos ya que parte de su ciclo lo pasan en el suelo expuestos al ambiente.

9. CONCLUSIONES GENERALES

La utilización de *Allium cepa* tuvo el 50% de eficacia, aunque esta se considera baja, no tuvo efectos adversos sobre los parámetros productivos y hematológicos en ovinos en crecimientos estabulados con dieta de mantenimiento, resaltando un punto importante puesto que no produce contaminación de suelos, agua y alimentos, y no deja residuos en carne por lo que no tiene tiempo de retiro.

Se identificó por PCR *Haemonchus contortus*, se encontró diversidad genética e identidad con aislados que presentan resistencia a tratamientos antihelmínticos en otros países, y por las características climáticas de la zona de donde se obtuvieron las muestras de *Haemonchus contortus* para este estudio los resultados sugieren que además de resistencia a antihelmínticos presenta resistencia a climas fríos. El diagnóstico molecular tiene ventajas prácticas, ya que puede guiar el manejo integral que se utilizará, reduciendo así las pérdidas económicas causadas por la resistencia antihelmíntica.

10. REFERENCIAS BIBLIOGRÁFICAS

Adamu, M., Oshadu, O.D., Ogbaje, C.I. 2010. Anthelminthic efficacy of aqueous extract of *Acanthus montanus* leaf against strongylid nematodes of small ruminants. African Journal of Traditional, Complementary and Alternative medicines. 7(4):279-285.

Ademola, I.O., Fagbemi, B.O., Idowu, S.O. 2005. Anthelmintic activity of extract of *Spondias mombin* against gastrointestinal nematodes of sheep: Studies *In vitro* and *In vivo*, Tropical Animal Health and Producction. (37):223-235.

Aktas, M., Altay, K., Dumanli, N. 2005. Development of a polymerase chain reaction method for diagnosis of *Babesia ovis* infection in sheep and goats. Veterinary Parasitology. 133:277–281.

Angulo, F.J., García, L., Alunda, J.M., Cuquerella, M., de la Fuente, C. 2010. Biological characterization and pathogenicity of three *Haemonchus contortus* isolates in primary infections in lambs. Veterinary Parasitology. 171(2010):99–105.

Barrère, V., Keller, K., Samson-Himmelstjerna, G., and Prichard, R.K. 2013. Efficiency of a genetic test to detect benzimidazole resistant *Haemonchus contortus* nematodes in sheep farms in Quebec, Canada. Parasitology International. 62(2013):464–470.

Bello, M.O., Olabanji, I. O., Abdul-Hammed, M. and Okunade, T.D. 2013. Characterization of domestic onion wastes and bulb (*Allium cepa L.*): fatty acids and metal contents. International Food Research Journal. 20(5):2153-2158.

Bhattacharjee, S., Sultana, A., Hasnan, M., Ariful, M., Ahtashom, M. Asaduzzaman, M. 2013. Analysis of the proximate composition and energy values of two varieties of onion (*Allium cepa* L.) bulbs of different origin: A comparative study. International Journal of Nutrition and Food Sciences. 2(5): 246-253.

Bidkar, A., Ghadiali, M., Chirag, P., Manoj, A., Deepa, S., Pallavi, A. 2012. Anthelmintic Activities of the Crude Extracts of *Allium cepa* Bulbs and *Elletatria cardomomum* Seeds. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 3(1):50-58.

Bisset, S.A., Knight, J.S., Bouchet, C.L.G. 2014. A multiplex PCR-based method to identify strongylid parasite larvae recovered from ovine faecal cultures and/or pasture samples. Veterinary Parasitology. 200(2014):117–127.

Borgers, M., De Nollin, S. 1975. Ultrastructural changes in *Ascaris suum* intestine after mebendazole treatment *in vivo*. Journal of Parásitology. 61:110-122.

Borgers, M., De Nollin, S., De Brabander, M., Thienpont, D. 1975a. Influence of the anthelmintic mebendazole on microtubules and intracellular organelle movement in nematode intestinal cells. American Journal of Veterinary Research. 36:1153-1166.

Borgers, M., De Nollin, S., Verheyen, A., Vanparija, O., Thienpont, D. 1975b. Morphological changes in cysticerci of *Taenia taeniaeformis* after mebenzazole treatment. Journal of Parásitology. 61:830-843.

Blackie, S. 2014. A review of the epidemiology of gastrointestinal nematode infections in sheep and goats in Ghana. Journal of Agricultural Science. 6(4).

Block, E. 1985. The chemistry of garlic and onions. Scientific American. 252(3):114-119.

Brard, C., Chartier, C. 1997. Quand suspecter une strongylose digestive chez les ovins et les caprins et conduite a tenir. Le Point Vétérinaire. 28:83-88.

Brasil, S.A.F.B., Nunes, R.L., Bastianetto, E., Drummond, M.G., Carvalho, D.C., Leite, R.C., Molento, M.B., Oliveira, D.A.A. 2012. Genetic diversity patterns of *Haemonchus placei* and *Haemonchus contortus* populations isolated from domestic ruminants in Brazil. International Journal for Parasitology. 42(2012):469–479.

Budischak, S.A., Hoberg, E.P., Abrams, A., Jolles, A.E., Ezenwa, V.O. 2015. A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. Molecular Ecology Resources. 15:1112–1119.

Campos, K.E.Y., Diniz, S., Cataneo, A.C., Faine, L.A., Alves, M. J., Novelli, E. L. 2003. Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: Dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. International Journal of Food Science Nutrition. 54:241-246.

Camurça-Vasconcelos, A.L.F., Bevilaqua, C.M.L., Morais, S.M., Maciel, M.V., Costa, C.T.C., Macedo, I.T.F., Oliveira, L.M.B., Braga, R.R., Silva, R.A., Vieira, L.S., Navarro, A.M.C. 2008. Anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes. Veterinary Parasitology. 154:167–170.

Chartier, C., Hoste, H. 2004. The use of anthelmintics in goats: efficiency and sustainability. Bulletin G.T.V. Hors-série. 125-130.

Chandrawathani, P. Jamnah, O. Waller, P.J. Larsen, M. Gillespie, A.T. Zahari, W.M. 2003. Biological control of nematode parasites of small ruminants in Malaysia using the nematophagous fungus *Duddingtonia flagrans*. Veterinary Parásitology. 117:173-183.

de Aquino Mesquita, M., Silva Júnior J.B.E., Panasso, A.M., de Oliveira, E.F., Vasconcelos, A.L., de Paula, H.C., Bevilaqua, C.M. 2013. Anthelmintic activity of *Eucalyptus staigeriana* encapsulated oil on sheep gastrointestinal nematodes. Parasitology Research. 112(9):3161–3165.

Garretson, P.D., Hammond, E.E., Craig, T.M., Holman, P.J. 2009. Anthelmintic resistant *Haemonchus contortus* in a giraffe (*Giraffa camelopardalis*) in Florida. Journal of Zoo and Wildlife Medicine. 40(1):131–139.

Githiori, J.B., Hoglund, J., Waller, P.J., Baker, R.L. 2003. The anthelmintic efficacy of the plant, *Albizia anthelmintica* against the nematode parasite *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice. Veterinary Parasitology. 116(1):23-34.

Githiori, J.B., Athanasiadou, S., Thamsborg, S.M. 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Veterinary Parasitology. 139(4):308-320.

González, G.R., Córdova, P.C., Torres, H.G., Mendoza, G.P., Arece J.G. 2011. Prevalence of gastrointestinal parasites in slaughtered sheep at a slaughterhouse in Tabasco, Mexico. Veterinaria México. 42(2):125-135.

González, R., Navarro, F., Arias, J., Gutiérrez, S., Zaragoza, M., Zaragoza, C. 2013. Morphological Description of *Haemonchus contortus* and *Mecistocirrus* *digitatus* of Sheep and Cattle in Tabasco, Mexico. Avances en Ciencias Veterinarias. 28(2):76-85.

Grade, J.T., Arble, B.L., Weladji, R.B., Van Damme, P., 2008. Anthelmintic efficacy and dose determination of *Albizia anthelmintica* against gastrointestinal nematodes in naturally infected Ugandan sheep. Veterinary Parasitology. 157:267–274.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M., Hoskin, S O. 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends in Parásitology. 22:253-261.

Ismail, A. M., Sedki, A. A., Abdallah, A. G. 2003. Influence of black seed, garlic and onion supplementation on reproductive performance in rabbits. Egypt Journal of Agricultural Research. 81:1193-1207.

Jackson, F., Coop, R.L. 2000. The development of anthelmintic resistance in sheep nematodes. Parásitology. 120:95-97.

Joshi, B.R., Kommuru, D.S., Terrill, T.H., Mosjidis, J.A., Burke, J.M., Shakya, K.P., Miller, J.E. 2011. Effect of feeding *Sericea lespedeza* leaf meal in goats experimentally infected with *Haemonchus contortus*. Veterinary Parasitology. 178(1-2):192–197.

Kaplan, R.M. 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends in Parásitology. 20:477-481.

Ketzis, J.K., Vercruysse, J., Stromberg, B. E., Larsen, M., Athanasiadou, S., Houdijk, J.G.M. 2006. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. Veterinary Parásitology. 139:321-335.

Kim, E.T., Kim, C.H., Min, K.S., Lee, S.S. 2012. Effects of Plant Extracts on Microbial Population, Methane Emission and Ruminal Fermentation Characteristics in In vitro. Asian-Australasian Journal of Animal Science. 25(6):806–811.

Kommuru, D.S., Whitley, N.C., Miller, J.E., Mosjidis, J.A., Burke, J.M., Gujja, S., Mechineni, A., Terrill, T.H. 2015. Effect of *Sericea lespedeza* leaf meal pellets on adult female *Haemonchus contortus* in goats. Veterinary Parasitology. 207(1-2):170-175.

Lanzotti, V. 2006. The analysis of onion and garlic. Journal of Chromatography A. 1112(2006):3–22.

Larsen, M. 1999. Biological control of helminths. International Journal of Parásitology. 29:139-146.

Lopes, S.G., Barros, L.B.G., Louvandini, H., Abdalla, A.L., Costa, L.J. 2016. Effect of tanniniferous food from *Bauhinia pulchella* on pasture contamination with gastrointestinal nematodes from goats. Parasites & Vectors. 9(102). DOI 10.1186/s13071-016-1370-3

López, O.A., González, R., Osorio, M.M., Aranda, E., Díaz, P. 2013. Gastrointestinal nematodes burden and prevalent species in hair sheep for slaughter. Revista Mexicana de Ciencias Pecuarias. 4(2):223-234.

MacKinnon, K.M., Zajac, A.M., Kooyman, F.N.J., Notter, D.R. 2010. Differences in immune parameters are associated with resistance to *Haemonchus contortus* in Caribbean hair sheep. Parasite Immunology. 32(7):484–493.

Mangkit, B., Thaenkham, U., Adisakwattana, P., Watthanakulpanich, D., Jantasuriyarat, C. and Komalamisra, C. 2014. Molecular Characterization of

Haemonchus contortus (Nematoda: Trichostrongylidae) from Small Ruminants in Thailand Based on the Second Internal Transcribed Spacer of Ribosomal DNA. Kasetsart Journal (Nat. Sci.). 48:740-758.

Mantawy, M.M., Aly, H.F., Zayed, N., Fahmy, Z.H. 2012. Antioxidant and schistosomicidal effect of *Allium sativum* and *Allium cepa* against *Schistosoma mansoni* different stages. European Review for Medical and Pharmacological Science. 16(3):69-80.

Meany, M., Allister, J., McKinstry, B., McLaughlin, K., Brennan, G.P., Forbes, A.B., Fairweather, I. 2007. *Fasciola hepatica*: ultrastructural effects of combination of tricabendazole and clorsulon against mature fluke. Parásitology Research.100:1091-1104.

Mederos, A., Fernández, S., VanLeeuwen, J., Peregrine, A.S., Kelton, D., Menzies, P., LeBoeuf A., Martin, R. 2010. Prevalence and distribution of gastrointestinal nematodes on 32 organic and conventional commercial sheep farms in Ontario and Quebec, Canada (2006-2008). Veterinary Parasitology. 170(3-4):244-52.

Mottier, L., Lanusse, C. 2001. Bases moleculares de la resistencia a fármacos antihelmínticos. Revista Médica Veterinaria. 82:74-85.

Mottier, L., Alvarez, L., Ceballos, C., Lanusse, C. 2006. Drug transport mechanisms in helminth parasites: Passive diffusion of benzimidazole anthelmintics. Experimental Parásitology. 113:49-57.

Moura, C.A., Dantas, S.F.J., Pacheco, A., Batista da Cunha, A., dos Santos Cruz, J., Scofield, A., Góes-Cavalcante, G. 2016. F200Y polymorphism of the β-tubulin isotype 1 gene in *Haemonchus contortus* and sheep flock management practices related to anthelmintic resistance in eastern Amazon. Veterinary Parasitology. 226(2016):104–108.

Nery, P.S., Nogueira, F.A., Martins, E.R., Duarte, E.R. 2010. Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. Veterinary Parasitology. 171(3-4):361-364.

Nunes, R.L., dos Santos, L.L., Bastianetto, E., Andrade, O., Aparecida, D., Brasil, S.A.F.B. 2013. Frequency of benzimidazole resistance in *Haemonchus contortus* populations isolated from buffalo, goat and sheep herds. Revista Brasileña de Parasitololgía Veterinaria Jaboticabal. 4(22):548-553.

Oliveira, L.M.B., Bevilaqua, C.M.L., Costa, C.T.C., Macedo, I.T.F., Barros, R.S., Rodrigues, A.C.M., Camurça-Vasconcelos, A.L.F., Morais, S.M., Lima Y.C., Vieira, L.S., Navarro, A.M.C. 2009. Anthelmintic activity of *Cocos nucifera* L. against sheep gastrointestinal nematodes. Veterinary Parasitology. 159:55-59.

Saddiqi, H.A. Iqbal, Z. Khan, M.N. Sarwar, M. Muhammad, G. Yaseen, M. Jabbar A. 2010. Evaluation of three Pakistani sheep breeds for their natural resistance to artificial infection of *Haemonchus contortus*. Veterinary Parasitology. 168(2010):141–145.

Salama, A.A., AbouLaila, M., Terkawi, M.A., Mousa, A., El-Sify, A., Allaam, M., Zaghawa, A., Yokoyama, N., Igarashi, I. 2014. Inhibitory effect of allicin on the growth of *Babesia* and *Theileria equi* parasites. Parasitology Research. 113(1): 275-283.

Sampath-Kumar, K. P., Debjit-Bhowmik, Chiranjib, Biswajit, Pankaj-Tiwari, 2011. *Allium cepa*: A traditional medicinal herb and its health benefits. Journal of Chemical Pharmacological Research. 2:283-291.

Sato, M.O., Sato, M., Chaisiri, K., Maipanich, W., Yoonuan, T., Sanguankiat, S., Pongvongsa, T., Boupha, B., Moji, K., Waikagul, J. 2014. Nematode infection among ruminants in monsoon climate (Ban-Lahanam, Lao PDR) and its role as food-borne zoonosis. Brazilian Journal of Veterinary Parasitology Jaboticabal. 1(23):80-84.

Silveira, R.X., Chagas, A.C.S., Botura, M.B., Batatinha, M.J.M., Katiki, L.M., Carvalho, C.O., Bevilaqua, C.M.L., Branco, A., Machado, E.A.A., Borges, S.L., Almeida, M.A.O. 2012. Action of sisal (*Agave sisalana*, Perrine) extract in the *in vitro* development of sheep and goat gastrointestinal nematodes. Experimental Parasitology. 131:162-168.

Tschuor, A.C., Riond, B., Braun, U., Lutz, H. 2008. Hämatologische und klinisch-chemische Referenzwerte für adulte Ziegen und Schafe. Schweizer Archiv Fur Tierheilkunde. 150(6):287–295.

Quiroz, H., Figueroa, J.A, Ibarra, F., López, M.E. 2011. Epidemiologia de enfermedades parasitarias en animales domésticos. Primera edición. México.

Ramos, F., Pires, P.L., de Souza, R.F., Zamperete, R.C., Pötter, L., Skrebsky C.Al., Sangioni, L.A., Flores, V.F.S. 2016. Anthelmintic resistance in gastrointestinal nematodes of beef cattle in the state of Rio Grande do Sul, Brazil. International Journal of Parasitology Drugs and Drug Resistance. 6(1):93–101.

Resende, N.M., Gazzinelli-Guimarães, P.H., Barbosa, F.S., Oliveira, L.M., Nogueira, D.S., Gazzinelli-Guimarães, A.C., Gonçalves, M.T., Amorim, C.C., Oliveira, F.M. Caliari, M.V. Rachid, M.A., Volpato, G.T., Bueno, L.L., Geiger, S.M., Fujiwara, R.T. 2015. New insights into the immunopathology of early

Toxocara canis infection in mice. Parasites & Vectors. 8(354). doi: 10.1186/s13071-015-0962-7

Rodríguez, V.R.I., Cob, G.L.A., Domínguez, A.J.L. 2001. Frecuencia de parásitos gastrointestinales en animales domésticos diagnosticados en Yucatán, México. Revista Biomedica. 12:19-25.

Roeber, F., Jex, A.R. and Gasser, R.B. 2013. Impact of gastrointestinal parasitic nematodes of sheep and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective. Parasites & Vectors. 6:153.

Rojas, H.S., Gutiérrez, S.I., Olivares P.J., Valencia A.M.T. 2007. Prevalencia de nemátodos gastrointestinales en ovinos en pastoreo en la parte alta del MPIO. De Cuetzala del Progreso, Guerrero-México," REDVET. Revista electrónica de Veterinaria. 8(9):1-7.

Saleheen, D., Ali, S.A., Yasinzai, M.M. 2004. Antileishmanial activity of aqueous onion extract *in vitro*. Fitoterapia. 75(2004):9–13.

Soulsby, E.J. 1982. Helminths, Arthropod´s and protozoa of domesticated animals. Balliere Tindall, London.

Souza, L.E., da Cruz, L. F., Teixeira-Neto, M.R., Albuquerque, G.R., Melo, A.D., Tapia, D.M. 2015. Epidemiology of Eimeria infections in sheep raised extensively in a semiarid region of Brazil. The Brazilian Journal of Veterinary Parasitology, 24(4):410-415.

Stevenson, L.A., Chilton, N.B., Gasser, R.B. 1995. Differentiation of *Haemonchus placei* from *H. contortus* (Nematoda: Trichostrongylidae) by the ribosomal DNA second internal transcribed spacer. International Journal of Parasitology. 25:483–488.

Prichard, R.K. 2002. Resistance Against Macrocyclic Lactones. En: Macrocyclic Lactones in Antiparasitic Therapy. Edit. Vercruysse, J.; Rew, R.S. CAB International. 164.

Terefe, G., Lacroux, C., Andreoletti, O., Grisez, C., Prevot, F., Bergeaud, J.P., Penicaud, J., Rouillon, V., Gruner, I., Brunel, J.C., Francois, D., Bouix, J., Dorchies, P., Jacquiet, P. 2007. Immune response to *Haemonchus contortus* infection in susceptible (INRA 401) and resistant (Barbados Black Belly) breeds of lambs. Parasite Immunology. 29(8):415-424.

Torres-Acosta, J.F.J., Mendoza-de-Gives, P., Aguilar-Caballero, A.J., Cuéllar-Ordaz, J.A. 2012. Anthelmintic resistance in sheep farms: Update of the situation in the American continent. Veterinary Parasitology. 189(2012):89–96.

Tschuor, A.C., Riond, B., Braun, U., Lutz, H. 2008. Hämatologische und klinisch-chemische Referenzwerte für adulte Ziegen und Schaf. Schweizer Archiv Fur Tierheilkunde. 150(6):287–295.

Van Wyk, J.A., Stenson, M.O., Van Der Merwe, J.S., Vorster, R.J., Viljoen, P.G. 1999. Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. Journal of Veterinary Research. 66:273-284.

Vargas-Magana, J.J., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A. Hoste, H., Chan-Pérez, J.I. 2014. Anthelmintic activity of acetone–water extracts against *Haemonchus contortus* eggs: Interactions between tannins and other plant secondary compounds. Veterinary Parasitology. 206(3–4):322-327.

Vercruysse, J., Rew, R. 2002. General Efficacy of the Macrocyclic Lactones to Control Parasites of Cattle. En: Macrocyclic Lactones in Antiparasitic Therapy. Edit. Vercruysse, J.; Rew, R.S. CAB International. 185-199.

Vidyashankar, A.N., Hanlon, B.M., Kaplan, R.M. 2012. Statistical and biological considerations in evaluating drug efficacy in equine strongyle parasites using fecal egg count data. Veterinary Parasitology. 185(1):45-56.

Vieira, L.S. 2008. Métodos alternativos de controle de nematóides gastrintestinais em caprinos e ovinos. Revista Tecnologia y Ciência Agropecuaria. 2(2):49–56.

Zintz, K., Frank, W. 1982. Ultrastructural modifications in *Heterakis spumosa* after treatment with febantel or mebendazole. Veterinary Parásitology. 10:47-56.