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Primer reporte de infección natural de Nematodirus filicollis en un ovino del trópico sub-húmedo mexicano

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Abstract:

Objective. The aim of the present study is to report for the first time the natural infection of a sheep with Nematodirus filicollis in the sub-humid tropics of Mexico. Materials and methods. Faecal samples were processed to identify and quantify faecal nematode eggs per gram of feces (EPG) using the flotation and modified McMaster techniques. Also, faecal cultures were made using the Corticelli-Lai technique. Results. The ewe showed a mixed infection with Trichuris spp. (100 EPG), Nematodirus (100 EPG) and other strongylida nematodes (1500 EPG). The egg size of Nematodirus spp. was 202±3 µ long and 97±2 µ wide. Larvae of Haemonchus, Trichostrongylus, Oesophagostomum and Nematodirus genera were identified from fecal cultures. Nematodirus larvae averaged 955±8.0 µ long and 28±0.5 µ wide. Whereas, the average length of the sheath tail was 233.4±41.6 µ. Conclusions. According to the morphological traits of eggs and larvae, it is concluded that the infection of the sheep corresponded to Nematodirus filicollis. It is concluded that N. filicollis is able to survive in the sub-humid tropics of Mexico and for the first time an acquired natural infection of this nematode in a sheep is reported.

Keywords: ewe, feces, larva, strongylida.

Resumen:

Objetivo. El presente estudio tiene por objetivo reportar por primera vez el caso de un ovino que adquirió la infección natural con Nematodirus filicollis en condiciones de clima tropical sub-húmedo. Materiales y métodos. Se tomó una muestra de heces que fue procesada para identificar y cuantificar la excreción de huevos de nematodos por gramo de heces (h/gh) mediante las pruebas de Flotación Centrifugada y McMaster. Resultados. Se identificaron larvas de Haemonchus, Trichostrongylus, Oesophagostomum y Nematodirus del género de heces. Las larvas de Nematodirus medían 955±8.0 µ de longitud y 28±0.5 µ de ancho. Además, el promedio de longitud de la cola de la sheath era de 233.4±41.6 µ. Conclusiones. Según los caracteres morfológicos de los huevos y larvas, se concluyó que el ovino estaba infectado con Nematodirus filicollis. Se concluye que N. filicollis es capaz de sobrevivir en las condiciones climáticas sub-húmedas de México y que por primera vez se reporta una infección natural de este nematodo en un ovino.

Keywords: oveja, heces, larva, strongylida.

Author notes

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orden strongylida (1500 h/gh). El tamaño de los huevos del Nematodirus fue de 202±3 µ de largo y 97±2 µ de ancho. En el cultivo de larvas se identificaron los géneros Haemonchus, Trichostrongylus, Oesophagostomum y Nematodirus. La longitud promedio de las larvas de Nematodirus fue de 955±8.0 µ y ancho de 28±0.5 µ, mientras que la longitud promedio de la cola de la vaina fue de 233.4±41.6 µ. **Conclusiones.** Con base en las dimensiones de las larvas y sus características morfológicas se reporta que la infección del ovino fue por Nematodirus filicollis. Se concluye que N. filicollis es capaz de sobrevivir en las condiciones del clima tropical sub-húmedo en México y se reporta por primera vez el caso de un ovino que adquirió la infección natural con este nematodo. **PALABRAS CLAVE:** borrega, heces, larva, strongylida.

**INTRODUCTION**

The genus Nematodirus Ransom, 1907 corresponds to nematodes of the small intestine of ruminants that have a direct life cycle and are members of the subfamily Nematodirinae (Nematoda: Trichostrongyloidea). The genus Nematodirus is composed of more than 45 species and many are distributed in ruminants mainly from the United Kingdom, Norway, New Zealand, Canada and the United States of America (1, 2).

In the United Kingdom, N. battus produces the death of thousands of lambs annually. In sheep herds, morbidity and mortality can reach 50-100% and 5-20%, respectively, with an impact on the welfare and economy of the farms (3).

In a phylogenetic study conducted by Hoberg (4) he showed that the Nematodirinae subfamily originated in the Holarctic with primary distributions determined through the Beringia. The stages of development of free life have adapted to extreme weather conditions, being relatively resistant to low temperatures and drying (4).

The genus Nematodirus (eg, N. battus, N. filicollis) has been mainly restricted to temperate regions of the northern hemisphere (3,5). In order for the third stage larvae (L3) to emerge, the embryoed egg must be exposed to low temperatures (close to 0 °C) for a prolonged period, followed by an increase in temperature above 10°C (6). However, N. battus has been reported in Sicilia, Italy (7), under conditions where the minimum temperature in winter sporadically reaches <11°C in this Mediterranean island. This adaptation to various temperate climates serves to concentrate the infecting larvae in the spring when there are susceptible lambs, limiting the reproduction of this nematode to one generation per year (8).

In Mexico, the genus Nematodirus has only been reported in sheep from regions with subtropical and temperate climates (9,10,11), where climatic conditions are favorable for the development of infective larvae and allows for the infection of vulnerable sheep. So far the genus Nematodirus has not been reported as a natural parasite of sheep in tropical climates in Mexico, for this reason this study reports for the first time, the case of a sheep that acquired the natural infection with Nematodirus spp. in sub-humid tropical climate conditions.

**MATERIALS AND METHODS**

**Design.** The report corresponds to a case study, with epidemiological circumstantial evidence related to its occurrence. In the Veterinary Parasitology laboratory of the Campus of Biological and Agricultural Sciences of the Universidad Autónoma de Yucatán (CCBA-UADY), the feces of a 24-month-old female sheep of the Katahdin breed were registered for the diagnosis of gastrointestinal parasites. The sample came from a sheep farm located in the municipality of Mérida in the state of Yucatán, Mexico. In the coproparasitoscopic examination the presence of eggs of Nematodirus spp. (Figure 1) was observed. Given this finding, we proceeded to visit the farm to carry out an epidemiological study and to obtain the possible origin of this nematode.
Study site. In the clinical history of the case the sheep was found to be born on a farm located in the municipality of Mérida in the state of Yucatán, Mexico. The state of Yucatán is located at a latitude of 19°31'-
21°38' N and a longitude of 87°22'- 90°25' W; It presents a sub-humid tropical climate with rains in summer. The maximum ambient temperature varies from 35 to 40°C and the minimum from 10 to 16°C, with an average room temperature of 27°C. Relative humidity varies from 65 to 90%, with an average of 80% and annual rainfall of 1,000 mm. Two annual seasons are present: rainy (from June to November) and dry season (from December to May) (12). The geomorphology belongs to the young carsotectonic system, classified as a geomorphological landscape of undulating structural plains with dissolution and denudation. Soils are mainly lithosol, rendzin and cambisol (13).

**Flock and sheep studied.** The farm has a total of 476 sheep, out of which 290 make up the breeding stock. As part of the genetic improvement program in November 2015, three sheep of the Katahdin breed from the municipality of Córdoba, Veracruz, Mexico were introduced into the farm. This municipality is located in the state of Veracruz, Mexico on the shore of the mountainous mountain range of the Sierra Madre Oriental and in the southern part of the mountainous region of the state of Veracruz. The climate of the region is temperate-humid with rains in the summer, average temperature of 18°C (6-35°C), annual rainfall of 1800 mm, with moderate cold in winter.

**Herd management.** All the sheep in the farm are kept in confinement and receive food in earthen pens. Juvenile animals are raised in high cages with a slat floor. The feeding consists in the administration of foliage of grasses (Pennisetum purpureum, Penissetum violaceum) and arboreal (Brosimum allicastrum, Guazuma ulmifolia and Leucaena leucocephala) grown in the same farm. The grass is fertilized with feces from the sheep in the farm. Additionally, sheep receive fresh water ad libitum and food supplements throughout the year. The sheep in the entire farm are dewormed with 1% ivermectin (0.2 mg/kg BW subcutaneously) and albendazole (4 mg/kg BW orally) every 3 months.
Clinical examination of sheep and herd. The physical examination of the sheep was performed (Figure 2) and pale mucous membranes (FAMACHA® of 3, score of 1-4), body condition of 3 (score 1-5) and diarrhea were found. The ovine positive to Nematodirus spp. was sampled again to confirm the infection along with 12 animals that remained in the same pen for the previous three months. Additionally, the three sheep from the municipality of Córdoba, Veracruz introduced in 2015 (suspected of introducing the nematode genus to the farm) were sampled with the intention of performing coproparasitoscopic tests.

![Figure 2](image.png)

**Figure 2.** Female sheep positive for *Nematodirus* spp. in the sub-humid tropics of Mexico.

Microscopic feces study. Of the positive sheep for Nematodirus spp. a 10 g stool sample was obtained directly from the rectum by using a clean polyethylene bag, which was stored under refrigerated conditions (4°C) in a refrigerator and transported to the Veterinary Parasitology laboratory of the CCBA-UADY for study. The sample was subjected to the Centrifuged Flotation and McMaster tests (14) for identification of helminth eggs and quantification of the elimination of eggs per gram of feces (EPG). Fifty eggs of the genus Nematodirus were measured with the aid of a micrometer adapted to an optical microscope (40 x). Two portions of the sample (3 g/each) were used to perform two larval cultures using the Corticelli-Lai method (14), the first portion immediately after harvesting was used to perform larval culture and the second was preserved for 21 days under refrigerated conditions (1-2°C) to later perform the culture of larvae in an incubator at 28°C. The larval L3 harvests of both cultures were carried out after 21 days. The classification of larvae L3 was determined by their morphology and size (8). In order to identify the species of the genus Nematodirus 100 larvae L3 were measured (width, total length and length of the tail) with a micrometer adapted to an optical microscope (10 x).

Database of the Veterinary Parasitology Laboratory of the CCBA-UADY. To know the existence of cases of Nematodirus spp. in ovines from the state of Yucatan, Mexico, the files of the Veterinary Parasitology Laboratory of the CCBA-UADY were reviewed from January 2008 to September 2016. Additional information was also obtained on positive cases such as the origin of the animals and the elimination of eggs per gram of feces.
RESULTS

Table 1 shows the sheep diagnosed with Nematodirus spp. at the laboratory of Veterinary Parasitology of the CCBA-UADY through centrifuged flotation and McMaster tests, from a total of 1272 sheep studied from January 2008 to September 2016. All positive animals came from areas of temperate climates that were introduced in flocks to the state of Yucatan. Only the sheep reported in the present case was born in the state of Yucatan and at the time of taking the fecal sample, it eliminated 100 EPG of Nematodirus spp.

<table>
<thead>
<tr>
<th>Location of the lamb in Yucatan</th>
<th>Year</th>
<th>Location of provenance</th>
<th>Climate</th>
<th>Nematodirus spp. (EPG)</th>
<th>Strongyloides*** (EPG)</th>
<th>Trichostrongylus spp. (EPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samahil</td>
<td>2008</td>
<td>Thuxcata</td>
<td>Temperate</td>
<td>50</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Samahil</td>
<td>2008</td>
<td>Thuxcata</td>
<td>Temperate</td>
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<td>Samahil</td>
<td>2008</td>
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<td>Temperate</td>
<td>150</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Samahil</td>
<td>2008</td>
<td>Thuxcata</td>
<td>Temperate</td>
<td>50</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Merida</td>
<td>2008</td>
<td>Estado de México</td>
<td>Temperate</td>
<td>50</td>
<td>750</td>
<td>0</td>
</tr>
<tr>
<td>Ciuochola</td>
<td>2009</td>
<td>Estado de México</td>
<td>Temperate</td>
<td>50</td>
<td>1400</td>
<td>0</td>
</tr>
<tr>
<td>Ciuochola</td>
<td>2009</td>
<td>Estado de México</td>
<td>Temperate</td>
<td>50</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Tropchanal</td>
<td>2010</td>
<td>Thuxcata</td>
<td>Temperate</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Merida</td>
<td>2015</td>
<td>Thuxcata</td>
<td>Temperate</td>
<td>500</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Merida*</td>
<td>2016</td>
<td>Yucatan</td>
<td>Tropical</td>
<td>100</td>
<td>1500</td>
<td>100</td>
</tr>
</tbody>
</table>

*Reported case, **order strongylids, EPG: eggs per gram of feces

All eggs of Nematodirus observed in the ovine feces presented thin cover, without color and conical ends (Figure 3). The size of the eggs was 202 ± 3 μ long and 97 ± 2 μ wide. The fact that the eggs are colorless, their morphology with conical ends and size ruled out the presence of Nematodirus battus as an infecting species.
The 12 sheep that remained in the pen with the positive ovine for Nematodirus spp. and the three sheep from the municipality of Córdoba, Veracruz did not eliminate eggs of Nematodirus spp.

Both the culture carried out without the previous cooling as well as the one that was carried out with one week cooling allowed the recovery of larvae of Nematodirus spp. The genera of nematodes identified in the larval cultures without the previous cooling of the faeces corresponded to Haemonchus (58.1%), Trichostrongylus (32.5%), Oesophagostomum (5.8%) and Nematodirus (3.4%). Likewise, the genera of nematodes identified in the larval cultures with the previous cooling of faeces corresponded to Haemonchus (59.0%) Nematodirus (24.4%), Trichostrongylus (15.1%) and Oesophagostomum (1.6%).

The larvae of Nematodirus spp. found in the sheep agree with the descriptions of other authors because they are easily recognizable due to their large size and length and the filamentous shape at the ends of the sheath tail. The average size of the larvae of the Nematodirus genus identified in the sheep was 955 ± 8.0 μ in length and 28 ± 0.5 μ in width. While the average length of the sheath tail was 233.4 ± 41.6 μ. The anterior tip of the larvae is rounded, with an oral cavity in the form of a straight tube and a filariform esophagus. There are eight large intestinal cells, well delimited with their respective nucleus. In the hind limb, the tail of the larvae have a fish tail shape that characterizes N. filicollis.
DISCUSSION

Several authors mention that for a natural infection of the Nematodirus genus to exist in sheep, most species require eggs to be exposed for prolonged periods at low temperatures (close to 0°C), followed by an increase in temperature higher than 10°C (6). However, Torina et al (7) report the natural infection of sheep with N. battus in Sicilia, Italy, where the minimum temperature in winter sporadically reaches <11°C. This last situation could have occurred in the farm studied since the natural infection occurred in a sub-humid tropical climate where temperatures of less than 10°C can only be recorded in January and February (12). In an experiment conducted by Van Dijk and Morgan (15), they compared the hatching requirements between N. battus isolates from Scotland and southwestern England and found that not all larval eggs require cold stimulation to produce infective stages. Likewise, Van Dijk and Morgan (3) mention that prolonged periods of high temperatures (25-30°C) do not necessarily affect the survival of N. battus eggs. It would be expected that high temperatures increase the mortality rates of infective larvae, but management practices such as the increase in animal load favors the infection of sheep with this nematode and allows its survival in extreme climates (3, 16).

Obtaining larvae L3 of N. filicollis from laboratory cultures with and without previous cooling of the feces, allows us to suppose that this genus does not necessarily require a cooling period and therefore can survive in the environmental conditions of the sub-humid tropic. Likewise, the establishment of the genus Nematodirus in other regions that are not temperate such as in Sicily, Italy (7), Hueytamalco, Puebla, Mexico (10) and in the present study demonstrates the geographical expansion of this nematode to places outside of areas where the eggs are cooled to produce infective larvae.

In a study conducted by Sánchez and Quiroz (11) 2% and 1% of sheep from Tlaxco, Tlaxcala, Mexico (cold weather 2603 m above sea level) were parasitized by N. battus and N. spathiger respectively. Most studies in Mexico report the presence of the Nematodirus genus without identifying the species of this nematode (9, 10). The present study is the first report for Mexico of N. filicollis in tropical climate; although it was not possible to obtain the adult parasites of N. filicollis because the infection occurred in a female registered ovine and the owner did not allow the sacrifice of the animal. In future studies, it will be necessary to obtain the adult stages and compare them morphometrically and genetically with other Nematodirus species and evaluate the nematode’s capacity to cause damage to the health of the sheep of the Mexican sub-humid tropics.

The low elimination of eggs (100 EPG) of N. filicollis in the ovine of this study and the climatic limitations of the Mexican tropics for the survival of the larval eggs of the nematode could explain the low transmission in the herd studied. In addition, the intensive production system that was handled in the study farm and the fertilization of the cutting grass with feces of animals of the same farm, including the feces of the animals that were introduced from Córdoba, Veracruz (animals suspected of introducing the nematode genus into the farm), could explain the transmission of this nematode in the Mexican tropics. In future studies it will be necessary to evaluate the capacity of N. filicollis to produce natural infections in different production and management systems for sheep raised in the Mexican tropics.

The mixed infection of N. filicollis with other nematodes of the strongylida order along with the genus Trichuris found in the study sheep could have contributed to the deterioration of the health of the animal and present a clinical picture of gastrointestinal nematodiosis, a condition frequently observed in sheep of the region (17).

In conclusion N. filicollis is capable of surviving the biogeographical and climatological conditions of the state of Yucatan, Mexico, and for the first time the case of a sheep that acquired the natural infection with N. filicollis in tropical sub-humid conditions is reported in Mexico.
ACKNOWLEDGEMENTS

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REFERENCIAS