DOI 10.2478/s11687-014-0243-6

VERSITA

HELMINTHOLOGIA, 51, 4: 288 – 292, 2014

First record of *Muellerius capillaris* (Nematoda, Protostrongylidae) in northwestern Argentina

V. H. SUAREZ[1*], E. A. BERTONI[1], J. F. MICHELOUD[1], M. M. CAFRUNE[1], A. E. VIÑABAL[1], J. QUIROGA ROGER[2], A. F. BASSANETTI[1]

[1]INTA - Estación Experimental Agropecuaria Salta, RN 68, km 172, 4403 Cerrillos, Salta, Argentina, [*]E-mail: *suarez.victor@inta.gob.ar*; [2]INTA – IPAF NOA, RN 9 km 1763, 4622 Posta de Hornillos, Jujuy, Argentina

Summary

Seven flocks of different composition of goat and/or sheep were evaluated for the presence of *Muellerius capillaris* (Mueller 1889) in sites of different climatic conditions and under different management practices in northwestern Argentina. The nematode was recovered and identified in four goat flocks and two mixed goat and sheep flocks. Three goat flocks and both mixed goat and sheep flocks were located in Lerma Valley (Salta), with the other goat flock located in Quebrada de Humahuaca (Jujuy). *M. capillaris* was not detected in the Puna of Jujuy (3500 m a.s.l.). First-stage larvae were recovered from fecal samples using the Baermann technique. Lungs from six goats revealed numerous small pulmonary nodules and areas of emphysema, as well as a small number of *M. capillaris* adults. This is the first report of *M. capillaris* in Argentina.

Keywords: *Muellerius capillaris;* goats; sheep; northwestern Argentina

Introduction

Livestock production in valleys and gorges in northwestern Argentina relies heavily on small ruminants, mostly goats, which are the main income for small-scale producers of milk and meat, along with family agriculture. Therefore, diagnosing and preventing sanitary problems of small ruminants is critical for determining competitiveness in these systems.

The protostrongylid nematode *Muellerius capillaris* (Mueller 1889) is a roundworm that parasitizes the lungs of goats and sheep, and other wild ruminants in several regions worldwide. In some areas, such as the Mediterranean basin, its prevalence reaches almost 100 % of adult individuals, especially goats, which exhibit a higher prevalence than sheep. In Eurasia and North Africa, mixed infections with other lung nematodes are frequent (Cabaret, 1984).

This lungworm is found in the bronchioles and small airways, as well as the pulmonary alveoli and subpleural tissue. Although the pathogenic effects of *M. capillaris* are low compared to those of other nematodes (Suárez, 2007), cases of respiratory difficulties, chronic bronchopneumonia (Berrag & Cabaret, 1996), and interstitial pneumonia have been mentioned. This species has also been reported as a predisposing agent for bacterial infections and production losses (Pandey *et al*., 1984; Thomson *et al*., 2000).

Adults of *M. capillaris* are ovoviviparous, laying eggs that hatch into first-stage larvae (L1) after migrating from the nodules to the airways. From the airways, they pass through the digestive tract and are shed in the feces. Due to their indirect life cycle, once in the external environment, L1 must find an intermediary host, such as numerous mollusk species of the genera *Helix* and *Deroceras* (Cabaret, 1981). Mollusks in contact with L1 become infected and develop into L2 (8 days) and then into L3 (15 days). Small ruminants become infected by ingesting the small mollusks contaminated with L3 or directly by feeding on infested grasses. After migrating to the lungs, the lungworms penetrate the alveolar spaces and induce the formation of a granulomatous nodule. During the prepatent period (3 – 5 weeks to some months) L3 develop into L4 and then into adult forms capable of reproducing and lay eggs (Cabaret, 1984).

In the Americas, *M. capillaris* has been reported in the United States, Canada, Brazil and Chile (Nimmo, 1979; Pybus & Shave, 1984; Machado & Lima, 1988; Alcaino & Gorma, 1999), with no records in Argentina to date. Here, we provide the first record of *M. capillaris* in goats and sheep in northwestern Argentina.

Materials and methods

The samples analyzed were collected from animals raised in Lerma Valley (Salta province) and in Quebrada de Humahuaca and the Puna (Jujuy province). The climate in Lerma Valley is temperate, typical of highland valleys

Table 1. Sampling data, type of analysis and prevalence of recovered *Muellerius capillaris* L1 for each flock and location

Flock and Location	Sampling date	Analysis Type	N° of samples	N° of pools	N° of positive samples	Mean prevalence (%)
A Lerma Valley	Dec 12 Jul 13 Aug 14	individual	69 goat	-	60	86.9
B Lerma Valley	Aug 13 Dec 13	individual	18 goat 15 sheep	-	13 5	54.5
C Lerma Valley	Sept 13 Sept 14	pool	17 goat 11 sheep	3 5	0 4	-
D Lerma Valley	Mar 13 Apr 13 May 13 Jul 13	individual	174 goat	15	3	-
E Lerma Valley	Apr 13	pool	12 goat	1	1	-
F Quebrada de Humahuaca	Jul 13 Aug 13 Dec 13	Individual	69 goat	19	8	-
G The Puna	Nov10 Feb 11 Apr 11	pool	96 sheep	10	0	-

(1050 m a.s.l.) in northwestern Argentina. Precipitation is concentrated in the summer, with a dry period from April to November; mean annual precipitation ranges from 700 – 800 mm in the central and southern portions of the valley to 1000 mm in the northwestern parts. Mean annual temperature is 17 °C (maximum: 36 °C; minimum: -6 °C), with a relative environmental humidity between 20 and 80 %. Quebrada de Humahuaca is located at 2200 m a.s.l., whereas the Puna ecoregion at above 3500 m a.s.l. The study area in both regions has a seasonal precipitation regime ranging between 180 and 250 mm, distributed from December to March (austral summer) with a mean temperature of 7.1 to 14 °C and great daily thermal amplitude.

Samples were taken from seven flocks (labeled A, B, C, D, E, F and G). Flocks A, B, C, D, and E were located in Lerma Valley (Salta), while flocks F and G were located respectively in Quebrada de Humahuaca and the Puna (Jujuy). Flocks A, B and C were subjected to extensive management, with flock A including only goats and flocks B and C including goats and sheep. All three flocks were located in the northern portion of Lerma Valley, with flock A being located in the environment with the highest precipitation in the valley. Flocks D, E and F were composed only of goats and subjected to a more intensive management, with irrigated pastures and annual crops, and stabling periods. Finally, flock G, which included only sheep and was subjected to extensive management on pastures. The genetic origin of the sampled goats was Criollo in B, C and F, and crosses of Criollo with dominance of Saanen in A, D and E. Sheep of flocks B and C were Criollo breed and G sheep were Corriedale and Merino breeds.

Fecal samples were obtained from adult individuals of all the seven flocks; number of individuals, pool numbers (mixture), and sampling dates for each flock are detailed in Table 1. In addition, samples were collected from young goats (ranging between six and eleven months old) of flocks D and F in November (n=15) and December (n=20) 2013 in order to perform two pools.

The lungs of individuals sacrificed for consumption were examined: four goat lungs in flock A (two in December 2012, one in January 2013 and one in January 2014); two goat lungs in flock B, (one in August 2013 and the other in January 2014); four sheep lungs in flock G (two in February 2011 and two in October 2011). The presence of L1 was observed using Baermann technique (Suarez, 1997), which has been shown to be efficient in recovering lungworms (Papadopoulos *et al*., 2004). This technique was performed both in individual samples and in pools of several individual samples. L1s were identified using the morphological characters described by Van Wyk *et al*. (2004). Adult forms were recovered in two steps. First, the lungs were palpated in order to detect the presence of nodules; then they were dissected off to find adult worms. Parasites were extracted from the tissue by gentle compression between two glass sides, and then carefully teased the parasites away from the tissues with thumb forceps. Then, to collect the remaining adult worms, the nodules were cut into several pieces, submerged in warm water (37 °C), and

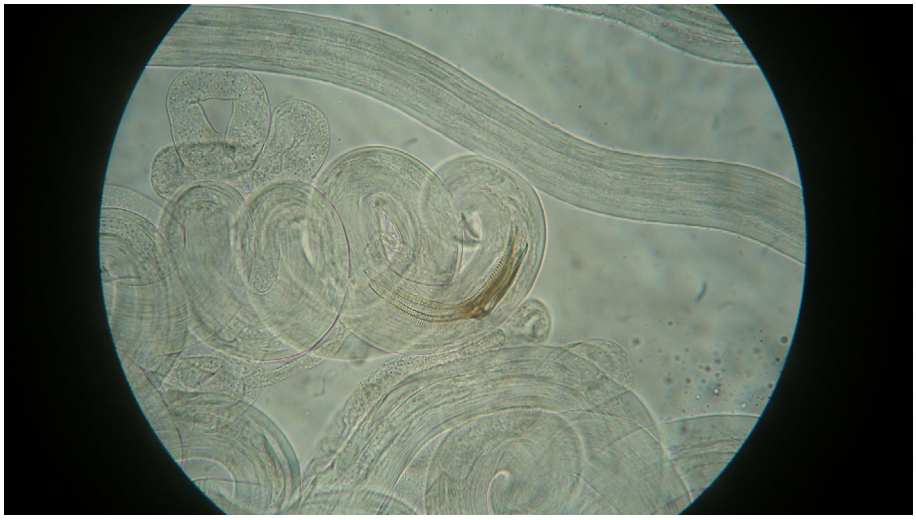

Fig. 1. Adult male of *Muellerius capillaris*

left overnight in funnels of plastic with a tube at the end. Finally, decanting worms at the bottom of the tube were recovered. Adults were identified following Boev (1975).
Several portions of lung tissue were collected from the sites where macroscopic lesions were observed. They were processed using the classical histological techniques of paraffin embedding, and were then sectioned (5 µm) and stained with hematoxilyn-eosin.
Data between goat and sheep faecal samples, at a level of significance of 5 %, were analysed by Chi-square test.

Results

The results of L1 recovery from fecal samples are shown in Table 1. Prevalence of L1-positive feces in flock B was higher (Chi 4.99; $p<0.03$) in goats (66.6 %) than in sheep (40 %); by contrast, in flock C, positive fecal pools were found only in sheep. Pooled samples collected from young goats of flocks D and F were negative. Adults (9 females and 2 males) and L1 of *M. capillaris* were recovered from lung nodules (Figs. 1 and 2).
In all cases of goat lungs, the observed macroscopic lesions included multiple subpleural 3 – 15 mm nodules, frequently coalescing. They were grayish-yellow and, in some cases, greenish. Sectioned nodules appeared solid and dry. All the lesions were mostly concentrated in the caudal lobules, mainly on the dorsal side. Histological analysis showed areas of bronchointerstitial pneumonia characterized by the presence of larval and adult stages of the intralesional parasite (Fig. 3). Adult stages were characterized by the presence of coelomic cavity and muscles, intestine, and in some cases, uterus full of eggs. An abundant mixed inflammatory infiltrate was observed in the alveolar septa surrounding these parasite accumulations, with a prevalence of lymphocytes, monocytes and occasionally some eosinophils. Giant cells were observed surrounding some larval stages, and a greater eosinophil concentration. Finally, hyperplasia of smooth muscle and an abundant mixed exudate were observed in some bronchioli.

Fig. 2. First-stage larvae of *Muellerius capillaris*

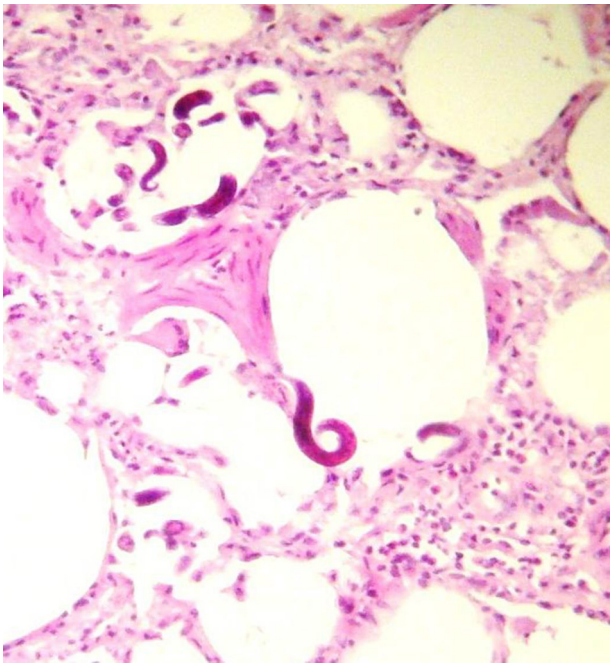

Fig. 3. Lung alveolar septa with first-stage larvae of *Muellerius capillaris*

Discussion

The recovery of L1 and adult forms of *M. capillaris* from samples collected from several flocks of goats and sheep in Salta and Jujuy, Argentina, on different dates and locations confirms for the first time the presence of this protostrongylid in the country. Previously, only *Dictyocaulus filaria* was recovered from small ruminant lungs (Suarez, 2007) and one report of an undescribed protostrongylid nematode from the pampas deer was cited (Carreno *et al*., 2012). Although it was possible to estimate the prevalence of animals infected with *M. capillaris* L1 only in two flocks (A and B, Table 1) where individual samples were analyzed, the results of the pooled samples suggest that the presence of *M. capillaris* was very variable among flocks and ecological zones. This variability was also observed in other regions, where prevalence ranged between 12 % and 80 % (Goncalves *et al*., 1980; Cabaret, 1986; Alemu *et al*., 2006; López *et al*., 2011).

The differences observed within the climatic environment of Lerma Valley (greater prevalence of *M. capillaris* in flocks A and B) might be due to the higher environmental humidity in the sites where these flocks are kept than in the location of flocks C, D and E. Higher humidity would provide a more favorable condition for the development of intermediate hosts. However, *M. capillaris* was also recovered from an arid environment, although with lower prevalence, such as flock F in Quebrada de Humahuaca. The more intensive exploitation regime of F might have provided higher chances of host infection. In Zaragoza, also an arid region, Uriarte *et al*. (1985) observed a greater prevalence of *M. capillaris* (55.2 %) in animals grazing on irrigated pastures than in animals present in non-irrigated pastures (37.9 %).

Regarding the difference in the presence of *M. capillaris* among host species, surprisingly the pools of feces from sheep were positive only in flock C. These findings are not consistent with results reported in the literature, which indicate higher susceptibility of goats as a host of *M. capillaris* (Cabaret, 1984), but such difference is probably due to a small sample size taken only on two dates. By contrast, *M. capillaris* was not detected in the arid Puna of Jujuy, at (3500 m a.s.l.), an area subjected to an extensive sheep management.

Pool samples taken from young goats in D and F were negative, which is consistent with previous results reporting that *M. capillaris* is much more abundant in adult goats and sheep (Cabaret, 1984). Both macroscopic lesions and their histopathological description coincide with previous reports (Caswell & Williams, 2007).

Although the present findings confirm the presence of *M. capillaris* in goat and mixed goat and sheep flocks in Lerma Valley (Salta) and Quebrada de Humahuaca (Jujuy), further research is necessary to elucidate which species of snails and slugs are associated with this nematode's life cycle, which factors favor its development, and how these and other management factors affect seasonal abundance and probable harmful effects of *M. capillaris* in the different regions of northwestern Argentina.

References

ALCAINO, H., GORMAN, T. (1999): Parásitos de los animales domésticos en Chile. *Parasitol. Día*, 23 (1 – 2): 33 – 41. DOI: 10.4067/S0716-07201999000100006

ALEMU, S., LEYKUN, E. G., AYELET, G., ZELEKE, A. (2006): Study on small ruminant lungworms in northeastern Ethiopia. *Vet. Parasitol*., 142: 330 – 335. DOI 10.1016/j.vetpar.2006.07.008.

BERRAG, B., CABARET, J. (1996): Impaired pulmonary gas exchange in ewes naturally infected by small lungworms. *Int. J. Parasitol*., 26: 1397 – 1400. DOI: 10.1016/S0020-7519(96)00132-4

BOEV, S. N. (1975): Protostrongylidae (Leiper, 1926). In: RYZHIKOV, M. M. (Ed) *Editions de l´Academic des Sciences d´ URSS*. Volume XXV. Moscow, pp. 160 – 166

CABARET, J. (1981): *Réceptivité des mollusques terrestres de la région de Rabat a l´infestation par les protostrongles dans les conditions expérimentales et naturelles*. Thèse Université Pierre et Marie Curie et Muséum National d´Histoire Naturelle, Paris

CABARET, J. (1984): Sheep and goats. Epidemiology of protostrongylids lungworms infections. *Int. Goat Sheep Res*., 2 : 142 – 152

CABARET, J. (1986): Repartition geographique des protostrongylides des ovins. Fréquence et importance de cette parasitose pulmonaire en Europe et en Afrique du Nord. *Epidémiol. Santé Anim*., 10: 61–72.

CARRENO, R. A., CAPOROSSI, D., BEADE, M. S., MARULL, C. A., UHART, M. M., MARKWARDT, D. D., NADLER, S. A. (2012): Discovery of an undescribed protostrongylid nematode from the endangered pampas deer (*Ozotoceros*

bezoarticus celer) in Argentina. *J. Wildl. Dis*., 48: 724 – 731. DOI: 10.7589/0090-3558-48.3.724
CASWELL, J. L., WILLIAMS, K. J. (2007): Respiratory system. In: GRANT MAXIE, M. (Ed) *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Fifth Ed. Sounders, Elsevier, Vol. 2, C. 5, pp. 626 – 627
GONCALVES, P. C., DOS SANTOS, J. O. P., UENO, H. (1980): *Muellerius capillaris* in sheep: occurrence and identification of adults. *Pesq. Agropec. Bras*., 15: 137 – 141
LÓPEZ, C. M., FERNÁNDEZ, G., VIÑA, M., CIENFUEGOS, S., PANADERO, R., VÁZQUEZ, L., DÍAZ, P., PATO, J., LAGO, N., DACAL, V., DÍEZ-BAÑOS, P., MORRONDO, P. (2011): Protostrongylid infection in meat sheep from Northwestern Spain: Prevalence and risk factors. *Vet. Parasitol*., 178: 108 – 114. DOI: 10.1016/j.vetpar. 2010.12.038
MACHADO, T. M. M, LIMA, W. S. (1988): *Muellerius capillaris* (Mueller, 1889) em caprinos no Estado de Minas Gerais. *Arq. Bras. Med. Vet. Zoot*., 40: 69 – 70
NIMMO, J. S. (1979): Six cases of verminous pneumonia (*Muellerius* sp.) in goats. *Can. Vet. J*., 20: 49 – 52
PANDEY, V. S., CABARET, J., FIKRI A. (1984): The effect of strategic anthelmintic treatment on the breeding performance and survival of ewes naturally infected with gastrointestinal strongyles and protostrongylids. *Ann. Rech. Vet*., 15: 491 – 495
PAPADOPOULOS, E., SOTIRAKI, S., HIMONAS, C., FTHENAKIS, G. C. (2004): Treatment of small lungworm infestation in sheep by using moxidectin. *Vet. Parasitol*., 121: 329 – 336. DOI: 10.1016/ j.vetpar 2004.02.026
PYBUS, M. J., SHAVE, H. (1984): *Muellerius capillaris* (Mueller, 1889) (Nematoda: Protostrongylidae): an unusual finding in Rocky Mountain bighorn sheep *(Ovis canadensis canadensis* shaw) in South Dakota. *J. Wildl. Dis*., 20: 284 – 288
SUÁREZ, V. H. (1997): Diagnóstico de las parasitosis internas de los rumiantes en la región de invernada. Técnicas e Interpretación. *Bol. Divulgación Técnica (INTA-Anguil, Argentina)*, 56, 50 p.
SUÁREZ, V. H. (2007): Epidemiología y control de los nematodes gastrointestinales en el oeste de la región pampeana. In: SUÁREZ, V. H., OLAECHEA, F. V., ROSSANIGO, C. E., ROMERO J. R. (Eds) *Enfermedades parasitarias de los ovinos y otros rumiantes menores en el cono sur de América*. Ediciones INTA, Arg., PT. 70, pp. 9 – 14
THOMSON, E. F., GRUNER, L., BAHHADY, F., ORITA, G., TERMANINI, A., FERDAWI, A. K., HREITANI, H. (2000): Effects of gastro-intestinal and lungworm nematode infections on ewe productivity in farm flocks under variable rainfall conditions in Syria. *Livest. Prod. Sci*., 63: 65 – 75. DOI 10.1016/S0301- 6226(99)00111-6
URIARTE, J., CABARET, J., TANCO, J. A. (1985): The distribution and abundance of parasitic infections in sheep grazing on irrigated or non-irrigated pastures in North-Eastern Spain. *Ann. Rech. Vét*., 16: 321 – 325
VAN WYK, J. A., CABARET, J., MICHAEL, L. M. (2004): Morphological identification of nematode larvae of small ruminants and cattle simplified. *Vet. Parasitol*., 119: 277 – 306. DOI 10.1016/j.vet.par.2003.11.012

RECEIVED APRIL 30, 2014

ACCEPTED AUGUST 18, 2014