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EFFICACY OF ANTHELMINTHIC CONTROL PROGRAMS AGAINST NATURAL *MUELLERIUS CAPILLARIS* INFECTION IN SHEEP IN THE NORTH-WEST OF SPAIN. EFFECT ON BLOOD GASES AND pH IN VENOUS BLOOD SAMPLES

LÓPEZ C.M., CIENFUEGOS S., DACAL V., VÁZQUEZ L., PANADERO R., FERNÁNDEZ G., DÍAZ P., LAGO N., DÍEZ-BAÑOS P. & MORRONDO M.P.*

Summary:

The field efficacy of a single dose treatment against natural *M. capillaris* infection using different anthelmintic drugs, extensively employed in ovine parasite control programs in Galicia (N.W. Spain), and the effect of protostrongylid infection on ovine respiratory functions, were evaluated. Five groups (n = 5) of ewes were used in this study; animals were treated with injectable ivermectin (0.2 mg/kg), levamisole (7.5 mg/kg) and albendazole (5 mg/kg) and monitored at 0, 7, 21, 42 and 63 days post-treatment (d.p.t.) by enumeration of the lungworm larvae per gram of faeces (l.p.g.) and determination of gas tension and pH in venous blood using an i-Stat portable clinical analyzer. No statistical difference was found either in larval elimination between untreated and treated groups or in the reduction in larval counts in all of the treated groups. A significant increase in partial oxygen tension (pO_2) and saturation (sO_2) between day 0 and 7 p.t. was observed in all treated animals. These values decreased significantly at day 21 to previous levels. There were no statistical differences in blood gases between uninfected and treated groups. We can conclude that under Galician field conditions, parasitic control programs are not totally effective against *M. capillaris* infection.

KEY WORDS: sheep, *Muellerius capillaris*, treatment, field efficacy, blood gases.

Résumé : EFFICACITÉ DE DIFFÉRENTS ANTHELMINTHIQUES VIS-À-VIS DE L'INFECTION NATURELLE PAR *MUELLERIUS CAPILLARIS* D'OVINS DANS LE NORD-OUEST DE L'ESPAGNE. EFFETS SUR LES GAZ DU SANG ET LE pH VEINEUX

L'efficacité sur le terrain d'un traitement à dose unique par différents anthelminthiques vis-à-vis de l'infection naturelle par M. capillaris *d'ovins en Galice (Espagne), ainsi que l'effet de cette infection sur la fonction respiratoire de ces ovins ont été évalués. Cinq groupes (n = 5) de brebis ont été utilisées dans cette étude. Les animaux ont été traités soit avec de l'ivermectine injectable (0,2 mg/kg), soit du lévamisole (7,5 mg/kg), soit de l'albendazole (5 mg/kg), et la charge parasitaire a été mesurée à 0, 7, 21, 42 et 63 jours post-traitement (j.p.t.) par la détermination du nombre de larves par gramme de fèces (l.p.g.) et par la mesure de la pO2, de la pCO2 et du pH dans le sang veineux à l'aide d'un i-Stat analyseur clinique mobile. Aucune différence statistique n'a été retrouvée pour ce qui concerne le nombre de larves présentes entre groupes traités et non traités. Des augmentations significatives de la pression partielle d'oxygène (pO_2) et de la saturation (SO_2) entre les jours 0 et 7 p.t. ont été observées chez tous les animaux traités. Au-delà de 21 j.p.t., ces valeurs sont revenues aux valeurs d'avant traitement. Nous pouvons conclure que, dans des conditions réelles en Galice, les programmes de contrôle des parasites ne sont pas efficaces contre l'infection par* M. capillaris.

MOTS CLÉS : *ovins,* Muellerius capillaris, *traitment, efficacité sur le terrain, gaz du sang.*

In Galicia (North-west of Spain) sheep are raised mainly in a semiextensive husbandry system. In this region, mild temperatures and environmental humidity are optimal for the survival of many important livestock parasites, such as small lungworms (Protostrongylidae). *Neostrongylus linearis*, *Muellerius capillaris, Cystocaulus ocreatus* and *Protostrongylus* sp. are the species parasitizing sheep in Galicia (Díez-Baños *et al.*, 1994), although a recent extensive survey carried out by Cienfuegos *et al.,* (2007) have shown *Muellerius capillaris* as the most frequent lungworm, with 98.2 % prevalence over the rest of the species and a mean of larval shedding of 67.4 ± 297.7 larvae per gram of faeces (lpg). Although clinical disease associated with protostrongylid infection is not very common in sheep, Valero *et al.* (1992) and Berrag & Cabaret (1996) found that heavy infections decreased carcass weights, increased levels of mortality and impaired pulmonary gas exchange.

In this study the field efficacy of three anthelmintic drugs (albendazole, levamisole and ivermectin) against *M. capillaris* natural infection, using protocols extensively used in ovine parasite control programs, was evaluated, together with the effect of natural infection by *M. capillaris* and the subsequent effect of treatment over pulmonary gas exchange under natural conditions.

MATERIALS AND METHODS

ANIMALS AND TREATMENT

In March 2008, 25 ewes from Lugo province were examined twice by means of the Baermann-Wetzel technique (Baermann, 1917; Wetzel, 1930) in order

* Parasitology and Parasitic Diseases. Animal Health Department. Lugo Veterinary Faculty. Santiago de Compostela University. 27002, Lugo, Spain.
Correspondence: Ceferino M. López.
Tel.: +34-82252231 - Fax: +34-82252195.
E-mail: c.lopez@usc.es

to confirm a natural pure infection by *M. capillaris*. Sheep grazed pastures around the farms during the day and were housed at night in strawed-floor stables. Sheep included in this study were ewes (> 3 years) that have not received any anthelmintic treatment during the last year (last treatment March-April 2007).
The ewes were ranked according to larval shedding before treatment. Within ranks, sheep were randomly assigned to four groups:
- Group 1 (n = 5), untreated control group.
- Group 2 (n = 5), treated with injectable ivermectin 1 % for ovine at the dose rate of 0.2 mg/kg body weight sc (Ivomec®, Merial Ltd. Essex, England).
- Group 3 (n = 5), treated with levamisole base (hydrochloride) 7.5 % at a dose rate of 7.5 mg/kg b.w. subcutaneously administered (Caliermisol®, Laboratorios Calier SA, Barcelona, Spain).
- Group 4 (n = 5), treated with albendazole 2.5 % at a dose rate of 5 mg/kg b.w. *per os* (Ganadexil®, INVESA Industrial Veterinaria S.A., Barcelona, Spain).
We also included a negative control group, composed by five ewes with no larval elimination in two successive samplings (Group 0).
After treatment, animals were maintained under field conditions, so that reinfections were possible.

Examination of faecal and blood samples

Faecal and blood samples were taken before treatment (day 0), and at 7, 21, 42 and 63 days post-treatment (d.p.t.). Faeces were collected directly from the rectum with plastic gloves and were kept cool until being analyzed by the Baermann-Wetzel technique in the same day.
Blood samples were collected from the jugular vein into Lithium-heparin vacuum tubes (BD Vacutainer®, Becton, Dickinson and Company) and were immediately analyzed for blood gas tension and pH, using the i-STAT portable clinical analyzer (i-STAT Corporation, East Windsor, USA). The following parameters were measured: pH, partial pressures of O_2 (pO_2) and CO_2 (pCO_2), oxygen saturation (sO_2), bicarbonate concentration (HCO_3), total CO_2 (TCO_2) and base excess (BE – amount of H^+ required to returned blood pH to reference value).

Data processing

Larval elimination was transformed to the logarithm of the count plus 1 to calculate geometric means. To assure homogeneity of sheep groups, larval counts in day 0 were compared using Kruskal-Wallis non parametric analysis. Differences in larval excretion at day 7, 21, 42 and 63 p.t. were analyzed using Mann-Whitney test to compare the treated groups with the positive untreated one. The significance in reduction of larval counts over time was tested with Friedman non parametric test for related samples.
The effect of the treatment over gas tension and pH in treated animals was analyzed with repeated measures ANOVA. "Repeated" contrast was introduced to determine if measures were significantly different from the adjacent sample. "Simple" contrast was used to compare between groups measures. All the analysis were realized with SPSS, Version 15.0.1, SPSS Inc., 1989-2006).

RESULTS

Treatment effect over larval elimination

The geometric mean of lpg and the statistical signification between untreated and treated groups are shown in Table I. All the animals of the positive control (G1) and treated groups (G2, G3 and G4) were positive previously to treatment. Neither statistical difference was found between untreated and treated groups during the study nor in the reduction in larval counts all through the study in all of the treated groups. All larvae recorded throughout the study were identified as *Muellerius capillaris*.

Treatment effect over pH and blood gas tension

Results of blood gas tension and pH of infected (G1-G4) and uninfected (G0) groups are shown in Table II. All treated groups (G2-G4) were considered as a whole (GT) because of the lack of statistical differences in

Animal group*	0 day	7 day	21 day	42 day	63 day
Group 1	5.8 (6,0)	10.8 (21.5)	19.0 (11.4)	23.1 (57.8)	18.5 (11.4)
Group 2	7.5 (3.8)	15.3 (10.3)	1.7 (0.0)	3.3 (0.0)	1.8 (0.0)
Group 3	10.8 (17.1)	28.4 (19.0)	26.0 (36.3)	13.1 (10.6)	5.4 (1.8)
Group 4	4.3 (4.5)	1.6 (0.4)	2.0 (0.3)	2.8 (0.5)	4.6 (2.2)

* G1: untreated group; G2: ivermectin treated group; G3: levamisole treated group; G4: albendazole treated group.

Table I. – Geometric Mean lpg and Median (between brackets) in the different groups of sheep.

Variable	Group	0 d.p.t.	7 d.p.t.	21 d.p.t.	42 d.p.t.	63 d.p.t.
pH	G1	7.41 ± 0.065	7.36 ± 0.084	7.38 ± 0.127	7.38 ± 0.090	7.38 ± 0.092
	GT	7.42 ± 0.053	7.44 ± 0.056	7.44 ± 0.044	7.43 ± 0.060	7.42 ± 0.058
	G0	7.41 ± 0.053	7.42 ± 0.022	7.43 ± 0.056	7.43 ± 0.026	7.42 ± 0.027
TCO_2	G1	26.2 ± 4.87	24.8 ± 5.97	28.0 ± 7.82	26.0 ± 6.44	26.0 ± 6.40
	GT	26.9 ± 2.85	27.0 ± 3.09	28.5 ± 2.72	28.7 ± 2.84	28.3 ± 2.87
	G0	25.8 ± 3.11	27.0 ± 2.55	27.8 ± 3.56	28.4 ± 2.88	27.2 ± 2.59
PCO_2	G1	38.6 ± 1.69	40.8 ± 7.05	39.4 ± 7.78	41.2 ± 6.81	40.6 ± 2.17
	GT	39.9 ± 6.23	38.0 ± 3.34	39.99 ± 5.12	40.2 ± 5.69	42.1 ± 5.00
	G0	38.6 ± 1.78	39.3 ± 1.80	38.6 ± 6.83	40.6 ± 4.56	39.9 ± 3.05
PO_2	G1	34.8 ± 4.49	46.0 ± 12.35	37.6 ± 5.59	37.6 ± 5.32	37.2 ± 9.44
	GT	34.2 ± 4.51	38.73 ± 5.92*	34.0 ± 5.96*	34.7 ± 5.18	35.6 ± 6.66
	G0	35.6 ± 10.53	37.2 ± 7.05	32.8 ± 5.80	32.2 ± 3.19	38.8 ± 6.69
HCO_3	G1	25.1 ± 4.75	23.6 ± 5.68	24.5 ± 4.30	24.9 ± 6.30	24.7 ± 6.14
	GT	25.6 ± 2.79	25.8 ± 3.05	27.29 ± 2.61	26.7 ± 2.23	27.1 ± 2.82
	G0	24.6 ± 3.17	25.7 ± 2.38	26.6 ± 3.40	27.2 ± 2.66	28.1 ± 5.85
BE	G1	0.4 ± 6.15	-2.0 ± 6.89	-0,8 ± 6.22	1.4 ± 5.27	-0.4 ± 7.54
	GT	1.1 ± 3.11	1.8 ± 3.70	3.3 ± 2.96	2.4 ± 2.95	2.5 ± 3.54
	G0	0.0 ± 4.00	1.2 ± 2.95	2.4 ± 3.97	3.0 ± 2.74	1.8 ± 2.77
sO_2	G1	67.0 ± 9.62	76.4 ± 13.01	69.0 ± 11.51	69.8 ± 7.33	67.4 ± 9.81
	GT	67.0 ± 7.99	73.7 ± 10.02*	65.3 ± 13.86*	66.9 ± 11.25	67.1 ± 11.69
	G0	69.6 ± 10.04	70.8 ± 10.03	63.0 ± 11.04	63.8 ± 6.91	73.0 ± 9.75

* Repeated Measures ANOVA (Repeated Contrast) $p < 0.05$ PO_2 and sO_2 7 days *vs* 0 days and PO_2 and sO_2 21 days *vs* 7 days.

Table II. – Arithmetic mean ± standard deviation of pH and blood gas tension of the uninfected (G0), positive control (G1) and all treated groups (GT) measured using the i-STAT portable clinical analyzer.

larval elimination. Using a repeated measures ANOVA with "Repeated" contrast, significant increase in partial oxygen tension (pO_2) and saturation (sO_2) between day 0 and day 7 p.t. ($F = 7.055$; $p = 0.019$ and $F = 7.076$, $p = 0.019$, respectively) were observed in GT. These values decreased significantly at day 21 ($F = 5.003$, $p = 0.042$ for pO_2 and $F = 5.342$, $p = 0.037$ for sO_2) to previous levels. The "Simple" contrast did not shown statistical differences between treated groups (GT) *vs* untreated parasitized control group (G1) or negative control group (G0) ($P > 0.05$).

DISCUSSION

None of the treatments that are usually employed in ovine parasite control programs in Galicia was completely effective against *M. capillaris*. Despite a temporary descent in larval elimination detected in all groups, at least one animal were eliminating larvae in faeces at every sampling time and most of them shed larvae at the end of the study (63 d.p.t.). Most anthelmintic treatments have shown an important lack of efficacy against protostrongylid lungworms, and in particular against *M. capillaris* (Bliss & Greiner, 1985; McCraw & Menzies, 1986; Helle, 1986; Díez-Baños *et al.*, 1995; Rehbein & Visser, 2002). Some of those authors found that *Muellerius* larvae reappeared in fecal samples in less than 60 days, even in animals under strict isolation. This reappearance might be explained by immature *Muellerius* forms, not affected by anthelmintic treatment, developing to maturity after destruction of the original adult population (McCraw & Menzies, 1986, 1988) or by the protection conferred by altered tissue surrounding that lungworm that is more pronounced than in other protostrongylid species (Rehbein & Visser, 2002). Recently, Papadopoulos *et al.* (2004) and Geurden & Vercruysse (2007) have obtained better results with moxidectin and eprinomectin, respectively, but in those cases larval elimination was studied for a short period of time after treatment, giving no time for the reappearance of larvae observed in other studies.

In Galicia, traditionally parasite control practices in ovine include a systematic single dose treatment in Spring and/or Autumn, mainly with benzimidazoles

(BZD), that generally it is designed to control gastrointestinal nematode infections. However, specific treatment for protostrongilyd infections would require higher dosage than these (Richard & Cabaret, 1992) or repeated treatments (McCraw & Menzies, 1986).

Blood gas tension and pH were obtained from venous samples with a portable clinical analyzer to reproduce the field clinical conditions. Though blood gases and pH are usually measured in arterial blood, according to García Alarcón *et al*. (2003) and Dascombe *et al*. (2007) venous values reflect correctly and can be used to evaluate the pulmonary gas exchange, particularly when values are used for comparative purposes (Verwaerde *et al.,* 2002).

The treated group (GT) was not significantly different from the positive or negative control groups. The differences found by Berrag & Cabaret (1996) between infected and uninfected ewes were due to the intense level of infection of the animals that exhibited typical clinical signs of intense bronchopneumonia. However, in our study treated animals increased significantly pO_2 and sO_2 levels at 7 d.p.t., either because of an improvement of the alveolar ventilation or the local perfusion (Berrag & Cabaret, 1996). In our opinion, the moderate intensity of infection of sheep in our study would provoke a slight chronic airway obstruction that may be compensated by increasing diaphragmatic force output. The temporary elimination of the larval population because of the different treatments would facilitate the effectiveness of the lungs pulling oxygen into the blood, observed as an increment in pO_2 and sO_2 levels. Later, at 21 d.p.t., pO_2 and sO_2 levels decreased significantly to levels previous to treatment, because of either the larval population retrieval or a natural compensation to normal values.

The Baermann migration technique is still considered the gold standard for the diagnosis of lungworm infections, although its sensitivity, in same cases, is ⩽ 90 % (Willard *et al*., 1988). Given the limits of the classical diagnosis (Traversa *et al*., 2008), there is a need for new immunological or molecular tools capable to provide a more reliable diagnosis and evaluation of the efficacies of anthelmintic treatments.

Despite the lack of efficacy of treatment against *M. capillaris* and the relatively low levels of infection, we detected a temporary improvement or rehabilitation of respiratory function after treatment. Regular clinical examinations through the trial did not reveal any abnormal respiratory signs; and when comparing infected and non infected animals it was observed that the low levels of infection did not impair significantly gas exchange. However, our results encourage us to develop new approaches with larger batches of animals to determine if blood gas analysis would be a valuable tool (easy to perform in the field) to detect an important protostrongylid infection.

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REFERENCES

Baermann G. Eine einfache Methode zur Auffindung von Ankylostomum-(Nematoden)-Larven in Erdproben. *Geneesk. Tijaschr. Nederl.-Indië*, 1917, *57,* 131-137.

Berrag B. & Cabaret J. Impaired pulmonary gas exchange in ewes naturally infected by small lungworms. *Int. J. Parasitol.*, 1996, *26*, 1397-1400.

Bliss E.L. & Greiner E.C. Efficacy of fenbendazole and cambendazole against *Muellerius capillaris* in dairy goats. *Am. J. Vet. Res.*, 1985, *46,* 1923-1925.

Cienfuegos S., Vázquez L., Dacal V., Pardo M., Fernández G., Lago N., Panadero R., Morrondo P. & López C. Estudio preliminar de las nematodosis broncopulmonares en el ganado ovino de Galicia. 10th Congreso Ibérico de Parasitología, Madrid, 2007, July 15-20.

Dascombe B.J., Reaburn P.R.J., Sirotic A.C. & Coutts A.J. The reliability of the i-STAT clinical portable analyser. *J. Sci. Med. Sport*, 2007, *10,* 135-140.

Díez-Baños P., Morrondo-Pelayo P., Carrillo-González B., López-Sández C. & Feijóo-Penela A. Relationship between the excretion of protostrongylid larvar in sheep in Northwest Spain and climatic conditions. *J. Helminthol.*, 1994, *68,* 197-201.

Díez-Baños P., Morrondo-Pelayo P., Feijóo-Penela A., Carrillo-González B. & López-Sández C. Evaluación de la aplicación del albendazol contra nematodos pulmonbares en ovinos en el noroeste de España. *Vet. Méx.*, 1995, *26,* 117-121.

García-Alarcón J., Valor-Sanz M.A., Corzo-Sierra J.L., Rodríguez-Jiménez A., Hernández-Alonso B. & Martín-Montes M. Gasometría venosa frente a gasometría arterial en pacientes con un patrón respiratorio ineficaz relacionado con la insuficiencia respiratoria crónica agudizada. *Enfermería clínica*, 2003, *13,* 73-80.

Geurden T. & Vercruysse J. Field efficacy of eprinomectin against a natural *Muellerius capillaris* infection in dairy goats. *Vet. Parasitol.*, 2007, *147,* 190-193.

Helle O. The efficacy of fenbendazole and albendazole against the lungworm *Muellerius capillaris* in goats. *Vet. Parasitol.*, 1986, *22,* 293-301.

McCraw B.M. & Menzies P.I. Treatment of goats infected with the lungworm *Muellerius capillaris*. *Can. Vet. J.*, 1986, *27,* 287-290.

McCraw B.M. & Menzies P.I. *Muellerius capillaris*: Resumption of shedding larvae in faeces following anthelmintic treatment and prevalence in housed goats. *Can. Vet. J.*, 1988, *29,* 453-454.

PAPADOPOULOS E., SOTIRAKI S., HIMONAS C. & FTHENAKIS G.C. Treatment of small lungworm infestation in sheep by using moxidectin. *Vet. Parasitol.*, 2004, *121,* 329-336.

REHBEIN S. & VISSER M. Efficacy of ivermectin delivered via a controlled-release capsule against small lungworms (Protostrongylidae) in sheep. *J. Vet. Med.*, 2002, *49,* 313-316.

RICHARD S. & CABARET J. Individual variations in efficacy of fenbendazole against the small lungworm *Muellerius capillaris* in dairy goats. *Small Rum. Res.*, 1992, *8*, 151-159.

TRAVERSA D., IORIO R. & OTRANTO D. Diagnostic and clinical implications of a nested PCR specific for ribosomal DNA of the feline lungworm *Aelurostrongylus abstrusus* (Nematoda, Strongylida). *J. Clin. Microbiol.*, 2008, *46*, 1811-1817.

VALERO G., ALLEY M.R. & MANKTELOW B.W. A slaughterhouse survey of lung lesions in goats. *N. Z. Vet. J.*, 1992, *40,* 45-51.

VERWAERDE P., MALET C., LAGENTE M., DE LA FARGE F. & BRAUN J.P. The accuracy of the i-STAT portable analyser for measuring blood samples and pH in whole-blood samples from dogs. *Res. Vet. Sci.*, 2002, *73,* 71-75.

WETZEL R. Zur Diagnose der Lungenwurminvasionen bei Rind und Schaf. *Dtsch. Tierärztl. Wschr.*, 1930, *38*, 49-50.

WILLARD M.D., ROBERTS R.E., ALLISON N., GRIEVE R.B. & ESCHER K. Diagnosis of *Aelurostongylus abstrusus* and *Dirofilaria immitis* infections in cats from a human shelter. *Am. J. Vet. Res.*, 1988, *192*, 913-916.

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