Efficacy of anthelmintic 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

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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 (pO₂) and saturation (SO₂) 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 utilisés 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 (pO₂) et de la saturation (SO₂) entre les jours 0 et 7 p.t. ont été observées chez tous les animaux traités. A l’issue de 21 j.p.t., ces valeurs sont revenue 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, traitement, 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). Neostongylus 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...
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 O₂ (pO₂) and CO₂ (pCO₂), oxygen saturation (SO₂), bicarbonate concentration (HCO₃⁻), total CO₂ (TCO₂) 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 significance 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

<table>
<thead>
<tr>
<th>Animal group*</th>
<th>0 day</th>
<th>7 day</th>
<th>21 day</th>
<th>42 day</th>
<th>63 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5.8 (6.0)</td>
<td>10.8 (21.5)</td>
<td>19.0 (11.4)</td>
<td>23.1 (57.8)</td>
<td>18.5 (11.4)</td>
</tr>
<tr>
<td>Group 2</td>
<td>7.5 (3.8)</td>
<td>15.3 (10.3)</td>
<td>1.7 (0.0)</td>
<td>3.3 (0.0)</td>
<td>1.8 (0.0)</td>
</tr>
<tr>
<td>Group 3</td>
<td>10.8 (17.1)</td>
<td>28.4 (19.0)</td>
<td>26.0 (36.3)</td>
<td>13.1 (10.6)</td>
<td>5.4 (1.8)</td>
</tr>
<tr>
<td>Group 4</td>
<td>4.3 (4.5)</td>
<td>1.6 (0.4)</td>
<td>2.0 (0.3)</td>
<td>2.8 (0.5)</td>
<td>4.6 (2.2)</td>
</tr>
</tbody>
</table>

* 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.
larval elimination. Using a repeated measures ANOVA with “Repeate
ed” contrast, significant increase in partial oxygen tension (pO2) and saturation (sO2) 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 pO2 and F = 5.342, p = 0.037 for sO2) 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**

* Repeated Measures ANOVA (Repeated Contrast) p < 0.05 PO2 and sO2 7 days vs 0 days and PO2 and sO2 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>0 d.p.t.</th>
<th>7 d.p.t.</th>
<th>21 d.p.t.</th>
<th>42 d.p.t.</th>
<th>63 d.p.t.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>G1</td>
<td>7.41 ± 0.065</td>
<td>7.36 ± 0.084</td>
<td>7.38 ± 0.127</td>
<td>7.38 ± 0.090</td>
<td>7.38 ± 0.092</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>7.42 ± 0.053</td>
<td>7.44 ± 0.056</td>
<td>7.44 ± 0.044</td>
<td>7.43 ± 0.060</td>
<td>7.42 ± 0.058</td>
</tr>
<tr>
<td></td>
<td>G0</td>
<td>7.41 ± 0.053</td>
<td>7.42 ± 0.022</td>
<td>7.43 ± 0.056</td>
<td>7.43 ± 0.026</td>
<td>7.42 ± 0.027</td>
</tr>
<tr>
<td>TCO2</td>
<td>G1</td>
<td>26.2 ± 4.87</td>
<td>24.8 ± 5.97</td>
<td>28.0 ± 7.82</td>
<td>26.0 ± 6.44</td>
<td>26.0 ± 6.40</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>26.9 ± 2.85</td>
<td>27.0 ± 3.09</td>
<td>28.5 ± 2.72</td>
<td>28.7 ± 2.84</td>
<td>28.3 ± 2.87</td>
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<tr>
<td></td>
<td>G0</td>
<td>25.8 ± 3.11</td>
<td>27.0 ± 2.55</td>
<td>27.8 ± 3.56</td>
<td>28.4 ± 2.88</td>
<td>27.2 ± 2.59</td>
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<tr>
<td>PCO2</td>
<td>G1</td>
<td>38.6 ± 1.69</td>
<td>40.8 ± 7.05</td>
<td>39.4 ± 7.78</td>
<td>41.2 ± 6.81</td>
<td>40.6 ± 2.17</td>
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<tr>
<td></td>
<td>GT</td>
<td>39.9 ± 0.23</td>
<td>38.0 ± 3.34</td>
<td>39.99 ± 5.12</td>
<td>40.2 ± 5.69</td>
<td>42.1 ± 5.00</td>
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<tr>
<td></td>
<td>G0</td>
<td>38.6 ± 1.78</td>
<td>39.3 ± 1.80</td>
<td>38.6 ± 6.83</td>
<td>40.6 ± 4.86</td>
<td>39.9 ± 3.05</td>
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<tr>
<td>PO2</td>
<td>G1</td>
<td>34.8 ± 4.49</td>
<td>46.0 ± 12.35</td>
<td>37.6 ± 5.59</td>
<td>37.6 ± 5.32</td>
<td>37.2 ± 9.44</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>34.2 ± 4.51</td>
<td>38.7 ± 5.92*</td>
<td>34.0 ± 5.96*</td>
<td>34.7 ± 5.18</td>
<td>35.6 ± 6.66</td>
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<tr>
<td></td>
<td>G0</td>
<td>35.6 ± 10.53</td>
<td>37.2 ± 7.05</td>
<td>32.8 ± 5.80</td>
<td>32.2 ± 3.19</td>
<td>38.8 ± 6.69</td>
</tr>
<tr>
<td>HCO3</td>
<td>G1</td>
<td>25.1 ± 4.75</td>
<td>23.6 ± 5.68</td>
<td>24.5 ± 4.30</td>
<td>24.9 ± 6.30</td>
<td>24.7 ± 6.14</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>25.6 ± 2.79</td>
<td>25.8 ± 3.05</td>
<td>27.29 ± 2.61</td>
<td>26.7 ± 2.23</td>
<td>27.1 ± 2.82</td>
</tr>
<tr>
<td></td>
<td>G0</td>
<td>24.6 ± 3.17</td>
<td>25.7 ± 2.38</td>
<td>26.6 ± 3.40</td>
<td>27.2 ± 2.66</td>
<td>28.1 ± 5.85</td>
</tr>
<tr>
<td>BE</td>
<td>G1</td>
<td>0.4 ± 6.15</td>
<td>-2.0 ± 6.89</td>
<td>-0.8 ± 6.22</td>
<td>1.4 ± 5.27</td>
<td>-0.4 ± 7.54</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>1.1 ± 3.11</td>
<td>1.8 ± 3.70</td>
<td>3.3 ± 2.96</td>
<td>2.4 ± 2.95</td>
<td>2.5 ± 3.54</td>
</tr>
<tr>
<td></td>
<td>G0</td>
<td>0.0 ± 4.00</td>
<td>1.2 ± 2.95</td>
<td>2.4 ± 3.97</td>
<td>3.0 ± 2.74</td>
<td>1.8 ± 2.77</td>
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<tr>
<td>sO2</td>
<td>G1</td>
<td>67.0 ± 9.62</td>
<td>76.4 ± 13.01</td>
<td>69.0 ± 11.51</td>
<td>69.8 ± 7.33</td>
<td>67.4 ± 9.81</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>67.0 ± 7.99</td>
<td>73.7 ± 10.02*</td>
<td>65.3 ± 13.86*</td>
<td>66.9 ± 11.25</td>
<td>67.1 ± 11.69</td>
</tr>
<tr>
<td></td>
<td>G0</td>
<td>69.6 ± 10.04</td>
<td>70.8 ± 10.03</td>
<td>63.0 ± 11.04</td>
<td>63.8 ± 6.91</td>
<td>73.0 ± 9.75</td>
</tr>
</tbody>
</table>

* One 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 elimi-
nating 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 protoscolid 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 feca-
samples in less than 60 days, even in animals under
strict isolation. This reappearance might be explained
by immature *Muellerius* forms, not affected by anthel-
mintic treatment, developing to maturity after destruc-
tion of the original adult population (McCraw & Menzies,
1986, 1988) or by the protection conferred by altered
tissue surrounding that lungworm that is more pro-
nounced than in other protoscolid 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...
Despite the lack of efficacy of treatment against protostrongyloid 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 \( p_O_2 \) and \( S_O_2 \) 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 \( p_O_2 \) and \( S_O_2 \) levels. Later, at 21 d.p.t., \( p_O_2 \) and \( S_O_2 \) 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 \( \leq 90 \% \) (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 protostrongyloid infection.

**ACKNOWLEDGEMENTS**

The authors thanks to OVICA (Galician association of ovine and caprine breeders) and the veterinarians of the ADSG ACIVO for their collaboration in the realization of this study. This work was supported by the research project PGIDIT06RAG26101PR.

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