Variability in susceptibility to anthelmintics of the lungworm Muellerius capillaris first-stage larvae. Relationship to dairy-goats farms and previous exposure to febantel treatment
A. Kulo, C. Chartier, Jacques Cabaret

To cite this version:
A. Kulo, C. Chartier, Jacques Cabaret. Variability in susceptibility to anthelmintics of the lungworm Muellerius capillaris first-stage larvae. Relationship to dairy-goats farms and previous exposure to febantel treatment. Veterinary Research, 1994, 25 (6), pp.537-543. hal-02708251

HAL Id: hal-02708251
https://hal.inrae.fr/hal-02708251
Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Variability in susceptibility to anthelmintics of the lungworm *Muellerius capillaris* first-stage larvae. Relationship to dairy-goat farms and previous exposure to febantel treatment

A Kulo ¹, C Chartier ¹, J Cabaret ²*

¹ CNEVA, Station Régionale de Pathologie Caprine, 60, rue de Pied-de-Fond, BP 3081, 79012 Niort cedex;
² INRA, Station de Pathologie Aviaire et de Parasitologie, Unité d'Écologie des Parasites, 37380 Nouzilly, France

(Received 19 July 1993; accepted 24 June 1994)

**Summary** — Seven dairy-goat farms, located in central, western France, were studied in order to assess the variability in susceptibility of the lungworm *Muellerius capillaris* first-stage larvae (L₁) to 3 different anthelmintics in relation to farm origin, by means of motility tests. The motility tests were performed by mixing larval suspensions with pyrantel (PYR), thiabendazole (TBZ) or ivermectin (IVE) solutions (3 concentrations x 3 incubation durations). The same anthelmintic tests were repeated 7 and 21 d after febantel (probenzimidazole) treatment of the goats. Before the treatment of goats, the average ratio (x 100) of L₁ motility compared with control were very different for the 3 anthelmintics: 44, 30 and 59, respectively for TBZ, PYR and IVE. The ratios of L₁ motility were the same at days 7 and 21 after the treatment of goats. The susceptibility of L₁ to anthelmintics varied a great deal from 1 farm to another when goats had not been previously treated, whereas L₁s from the same farms were the same after treatment of goats. The occurrence of different motilities of L₁ with the anthelmintic tests from 1 farm to another should be related to the existence of different populations of *M capillaris*. The L₁ motilities were reduced after anthelmintic treatment of goats suggesting a temporary effect on populations of *M capillaris* females shedding L₁.

*nematode / dairy-goat / Muellerius capillaris / febantel*

Sept fermes caprines du centre ouest de la France ont été suivies, afin de déterminer si la sensibilité à 3 anthelminthiques des larves du premier stade (L1) du strongle pulmonaire *Muellerius capillaris* dépendait de leur ferme d’origine. Des tests fondés sur la mobilité des L1 ont été utilisés pour apprécier cette sensibilité. Les larves L1 ont été mises en suspension avec du pyrantel (PYR), du thiabendazole (TBZ), ou de l’invermectine (IVE) (3 concentrations x 3 durées d’incubation). Un test de sensibilité aux anthelminthiques des L1 a été pratiqué le jour du traitement des chèvres avec un probenzimidazole, le fèbantel, puis 7 et 21 j après. Avant traitement, les rapports moyens de mobilité des L1 comparés au témoin (x100) étaient très différents selon les anthelminthiques : 44,30 et 59 respectivement pour les lots TBZ, PYR et IVE. Sept et 21 jours après le traitement des chèvres, ces rapports sont devenus proches. Les populations des L1 ont donc une variabilité importante de sensibilité aux anthelminthiques selon les fermes pour un premier contact avec les anthelminthiques, mais les différences sont réduites après un traitement des chèvres qui homogénéise la sensibilité des L1 aux anthelminthiques. Cette réduction de variabilité après un traitement des chèvres suggère une action du fèbantel sur les femelles de M capillaris, source des L1.

**nématoide / chèvre laitière / Muellerius capillaris / fèbantel**

**INTRODUCTION**

Dairy-goat farms in France are isolated from helminth transmission. The majority of animals are bought when the farm is created and only non-infected animals (young kids) are purchased later (Cabaret and Gasnier, unpublished data). The original populations of nematodes may be modified by management and anthelmintic treatments and thus strongly differ from one farm to another.

Benzimidazoles have been used for many years in goats, whereas ivermectin has been introduced more recently and only in dry goats (Cabaret *et al*, 1986). Resistance of digestive-tract strongyles to anthelmintics has been assessed using the ability of eggs to develop into larvae or on third-stage larvae (Presidente, 1985). Similarly, the susceptibility of adult small lungworms to anthelmintic could be assessed on first-stage larvae (L1) excreted in faeces. We have 3 categories of anthelmintics for *Muellerius capillaris* infection in goats: totally inefficient (pyrantel); very poorly efficient (thiabendazole); and moderately efficient (a probenzimidazole, fèbantel, several benzimidazoles, and ivermectin, see Cabaret *et al*, 1978, 1984; Bankov, 1981; Denev *et al*, 1986; McCraw and Menezes, 1986).

The motility of the small lungworm *M. capillaris* L1 is highly correlated with its infective ability towards their intermediate hosts land-snails (Cabaret, 1980). Motility is a good indicator of viability and could thus be used as a criterion in anthelmintic tests. The goals of present work were: i) to assess the diversity of susceptibility of L1 to 3 species of anthelmintics (classified as non-, very poorly and moderately efficient) in relation to farm origin, by means of motility tests; ii) to evaluate the influence of a previous exposure of goats to a moderately efficient probenzimidazole anthelmintic (fèbantel) treatment on subsequent L1 susceptibility to the 3 types of anthelmintics mentioned above; and iii) to estimate whether motility tests are reliable tools for assessing the efficacy of anthelmintic treatment or simply markers of differences between farms.

**MATERIALS AND METHODS**

*Dairy-goat farms and faecal samplings*

Seven dairy-goat farms located in central, western France were investigated. They utilized pasture throughout the year. Flock size ranged from
35 to 180 adult goats. The number and type of anthelmintic treatments were recorded during the year preceding the experiment (table I).

Faecal samplings were performed individually on 30-40 goats in each farm in the following spring; the goats had not been treated for at least 2 months when sampled. The goats were weighed and then treated on day 0 with febantel (5 mg/kg body weight), a probenzimidazole which has previously been tested against *M. capillaris* in sheep and goats (Bankov, 1981; Denev et al., 1986). Faecal samplings were carried out on days 0, 7, and 21 after treatment.

**First-stage larvae extractions and counts**

The first-stage larvae (L1) were extracted individually in baermann funnels from 3 g of faeces/goat (Cabaret et al., 1980). The mean number of L1 per gram of faeces (LPG) was assessed for each goat; the mean value and range for each farm on day 0 are shown in table I. The L1 of all goats from a farm were pooled and used for anthelmintic tests.

**Anthelmintic test on L1**

Three different anthelmintics were tested: poorly efficient thiabendazole (Thibenzole, MSD), inefficient pyrantel (Exhelm, Pfizer) and moderately efficient ivermectin (Ivermectin, MSD). They were chosen because they were all soluble in water and had different modes of action. The dose in 1 l of stock solution was calculated as follows: usual dosage (mg/body weight) x 50 kg (average weight of goats) / 5 (litres of blood). The concentrations of stock solution were thus 500, 200 and 2 ppm respectively for thiabendazole (TBZ), pyrantel (PYR) and ivermectin (IVE). The stock solutions were diluted to 10% and 1%.

The L1 were concentrated up to 2–6 larvae in 10 μl and were mixed with an equal volume of anthelmintic solutions and deposited in wells of microplates. Three samples of each anthelmintic were used. The L1s with anthelmintic solutions were incubated at 4°C during 1, 3 and 7 d. One control (L1 in tap water) was done for each dilution. Three replicates were used for each anthelmintic concentration-incubation time; 100–150 L1s were tested for the 3 replicates.
Motility rates were established as follows: i) microplates were removed from the 4°C refrigerator and left for 30–45 min at room temperature before examination; and ii) the motility ratio for a concentration was the mean percentage of motile L1 in the 3 replicates divided by the percentage of motile L1 in the control. These motility ratios (× 100) were the variables used throughout the study.

**Data processing**

Analysis of variance (Anova) and correspondence analysis (CA) were performed with a Stat-Itcf computer package (1988). In the latter, each axis corresponded to a linear combination of variables; these combinations were established in such a manner that the axes were totally unrelated (the axes were then perpendicular). Thus, the observed variables were combined to construct unrelated variables called axes. Data and observed variables were projected on the first plane constructed by axes 1 and 2; positively related variables were located on the same region of the plane.

**RESULTS**

**Between-farm susceptibility of *M* capillaris L1 to anthelmintics before and after treatment of goats with febantel**

The motility ratios of L1 are shown in table II. Before treatment the average ratios were 44, 30, and 59 for thiabendazole, pyrantel and ivermectin respectively. The motility ratio depended on farm and anthelmintic type, as well as concentration of anthelmintic and incubation duration (Anova: table III). The existence of an interaction between farm and anthelmintic showed that the L1 from each farm (or group of farms) responded in their own way. Coefficients of variation of L1 motility ratio were reduced after treatment of goats with pyrantel and thiabendazole, and remained similar with ivermectin (table II).

**Multivariate analysis of *M* capillaris L1 susceptibility to anthelmintics before and after treatment of goats with febantel**

As the variables were not independent, a multivariate analysis (CA) was used. The first used farms as variables; the responses to motility tests of L1 at d0 were the population responses (3 types of anthelmintics x 3 concentrations x 3 incubation durations, ie 27 test responses). The results obtained at d21 were introduced into the analysis as supplementary variables (their position is located on the graph, but they do not take part in the construction of axes and the relative position of farms, as established on data at d0). At d0, farms were distributed in several areas on the planes constructed with axes 1 and 2 (fig 1). The first group includes farms 2, 3 and 6 and the second, farms 4 and 5; farms 1 and 7 were isolated from the other farms. At d21 post-treatment, the farms showed less difference on the basis of L1 motility and were located on the same area of the plane constructed with axes 1 and 2.

In the second CA the anthelmintics were the variables; the aim was to characterize...
Table II. Average motility ratio of *M. capillaris* L1 in relation to farm, febantel treatment (5 mg/kg bodyweight) of goats, and anthelmintic used.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Thiabendazole</th>
<th>Motility* after test</th>
<th>Pyrantel</th>
<th>Ivermectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d0</td>
<td>d7</td>
<td>d21</td>
<td>d0</td>
</tr>
<tr>
<td>1</td>
<td>47</td>
<td>38</td>
<td>52</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>40</td>
<td>47</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>44</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>36</td>
<td>44</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>46</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>37</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>31</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Mean</td>
<td>44</td>
<td>39</td>
<td>47</td>
<td>30</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>28</td>
<td>13</td>
<td>20</td>
<td>70</td>
</tr>
</tbody>
</table>

* The motility ratio were assessed at 3 concentrations: 500, 50 and 5 ppm (thiabendazole); 200, 20 and 2 ppm (pyrantel); 2, 0.2 and 0.02 (ivermectin). The ratio at the 3 concentrations were averaged for each anthelmintic.

Table III. Motility ratio of *M. capillaris* L1 in anthelmintic tests before treatment of goats: relationship to farm origin, type of anthelmintic, concentration and incubation duration (4-way analysis of variance).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Degree of freedom</th>
<th>F-test value *</th>
<th>% of variability of motility ratio explained by variable a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
<td>6</td>
<td>18.35</td>
<td>11.5</td>
</tr>
<tr>
<td>Type of anthelmintic</td>
<td>2</td>
<td>49.92</td>
<td>10.5</td>
</tr>
<tr>
<td>Test concentration of anthelmintic</td>
<td>2</td>
<td>106.47</td>
<td>22.4</td>
</tr>
<tr>
<td>Incubation duration</td>
<td>2</td>
<td>36.07</td>
<td>7.6</td>
</tr>
<tr>
<td>Farm-anthelmintic</td>
<td>12</td>
<td>8.42</td>
<td>10.6</td>
</tr>
<tr>
<td>Within-group variation</td>
<td>48</td>
<td></td>
<td>5.1</td>
</tr>
</tbody>
</table>

* P < 0.01; a (between-group sum of squares x 100) / total sum of squares.
farms in relation to L1 motility according to test anthelmintic. The motilities at d0 were used actively in analysis whereas the motilities at d21 were used as supplementary data. The first axis (88% of inertia, statistically significant at less than $p < 0.05$) corresponded to decreasing L1 motility in relation to PYR whereas axis 2 (12% of inertia: not statistically significant) corresponded to motility according to TBZ and IVE tests (fig 2). One group of farms (2, 3, 6) had L1 whose motility was not depleted in contact with PYR at d0 but was reduced at d21. A second group of farms (4, 5) was equally motile L1 on d0 and d21. Farms 1 and 7 had higher L1 motility in relation with IVE and TBZ respectively.

DISCUSSION

The motility of L1, when in contact with anthelmintics, was very different from one farm to another. This might be explained by: i) innate resistance of some populations to one or several anthelmintics; and ii) acquired resistance to anthelmintics as already demonstrated in digestive-tract strongyles (Kerboeuf et al, 1988) in a neighbouring region. The latter explanation is not supported by historical records of treatments. Thiabendazole did not discriminate between farms, although benzimidazoles have been used between 0 to 5 occasions in 1990, whereas pyrantel significantly discriminated between farms although it has been scarcely used and is ineffective against *M. capillaris* infection. The first hypothesis seemed more probable: L1 maintained motility when in contact with selected anthelmintics by mere chance, possibly issued from genetic drift in isolated dairy-goat populations of *M. capillaris*. Modifications of ecological characteristics have been shown in digestive-tract strongyles of goat origin after 2 or 3 generations in experimental conditions (Gasnier and Cabaret, 1992) and a similar evolution might occur in isolated populations of *M. capillaris* in dairy-goat farms. It is also probable that the motility of L1 is not a good indicator of susceptibility of adult population to anthelmintics; this question has never been addressed, either for lungworms or digestive-tract strongyles, which have been extensively investigated for resistance to anthelmintics. The motility tests would then only be markers of genetic diversity in *M. capillaris* resulting either from genetic drift or selective pressure exerted in each farm.

The results of L1 motility tests were strongly modified after anthelmintic treatment of goats with the probenzimidazole febantel. The percentage of reduction of L1 excretion is largely dependent on the characteristics of goats and intensity of initial infection (Richard and Cabaret, 1992) but the remaining larvae were usually few and an apparent selection had been performed on *M. capillaris*. Diversity of the L1 motility test responses were reduced and the farms looked alike on the basis of L1 motility tests.
A reduced between-farm diversity in survival of L1 at various temperatures has also been recorded after treatment of goats (Kulo et al., 1994), indicating that the variability in susceptibility to anthelmintics is probably related to differences of biological characteristics. The remaining questions are the following. Is this subsequent homogeneity in motility tests long-lasting? How is L1 motility related to the biological capabilities of surviving larvae?

ACKNOWLEDGMENTS

This study was funded by the Conseil Regional Poitou-Charentes in France. We are also grateful to the Togo Government for financial support in the form of a PhD grant to A Kulo.

REFERENCES

Bankov DE (1981) Trials for therapy of Muellerius in goats. 9th International conference of WAAVP July 13-17, Budapest
Gasnier N, Cabaret J (1992) Evolution of ecological characteristics of one field isolate of Teladorsagia circumcincta (Nematoda) under laboratory conditions. 11th European Multicolloquium of Parasitology, September 7-11, The Hague, Netherlands, p 48