BETWEEN GOAT-FARM BIOLOGICAL VARIABILITY OF THE NEMATODE *MUELLERIUS CAPILLARIS* FIRST-STAGE LARVEA. INFLUENCE OF ANTHELMINTIC TREATMENT

KULO A.*, CHARTIER C.* and CABARET J.**

Summary :
Seven dairy-goat farms from the centerwest of France were investigated for morphology and ecology of first-stage larvae (L1) of *Muellerius capillaris* before and after treatment of goats with febantel, a probenzimidazole anthelmintic.The lengths, survivals at 20 and -20°C, and infectivity of L1 to intermediate host *Helix aspersa*, were different between farms. The between farms differences in survivals were reduced after treatment of goats with febantel. The observed between farm differences in L1 did not seem to be related to farm characteristics (intensity of treatments, susceptibility to febantel, and intermediate host species).

KEY WORDS : *Muellerius*. lungworm. first-stage larvae. temperature. goat.

Résumé : VARIABILITÉ BIOLOGIQUE INTER-FERMES CAPRINES DES LARVES DU PREMIER STADE DU NÉMATODE *MUELLERIUS CAPILLARIS*. INFLUENCE D'UN TRAITEMENT ANTHELMINTHIQUE.

Sept fermes caprines du centre-ouest de la France ont été étudiées pour la morphologie et l'écologie des larves de Muellerius capillaris *du premier stade (L1) avant et après traitement des chèvres avec du febantel, un anthelminthique probenzimidazole. La longueur, les survies à 20 et -20°C, et l'infestivité des L1 pour l'hôte intermédiaire* Helix aspersa, *sont différentes selon les fermes. Les différences inter-fermes, en ce qui concerne les survies, sont réduites après un traitement au fébantel. Les différences entre fermes des L1 ne semblent pas reliées aux caractéristiques de l'élevage (intensité des traitements, sensibilité au fébantel et espèces d'hôtes intermédiaires).*

MOTS CLES : *Muellerius. strongle pulmonaire. premier stade larvaire. température, chèvre.*

INTRODUCTION

Muellerius capillaris Müller, 1889 (Nematoda : Protostrongylidae) is the only species of lungworm recovered from dairy-goats in western France ; it is also highly prevalent (Cabaret *et al.* 1986 ; Chartier and Reche, 1992). The adult worms live in the lungs where eggs and first-stage larvae (L1) are excreted and then passed in the faeces. Survival of L1 in goat faeces had been studied (Cabaret *et al.*, 1991 ; Morrondo-Pelayo *et al.*, 1992) and desiccation of faeces was considered as a key factor at 20°C ; maintenance at temperatures over 30°C drastically reduced survivals. The length of L1 can vary from one place to another (see Boev, 1975) and thus could be an indicator of ecological diversity. Land snails serve as the intermediate hosts : the L1 enters the foot of the snail, and then after two moults, becomes third-stage larvae. Among land snails, juvenile *Helix aspersa* Müller 1774 might be used to monitor infectivity of L1 (Cabaret, 1992).

Dairy-goat farms do not exchange habitually goats and helminth populations are thus isolated and may evoluate in different ways in relation to general farm management (Cabaret and Gasnier, 1993) or anthelminthic treatments. The purposes of the present work were to measure the between farm variability regarding : i) the length of L1 and its value as a marker of biological variability, ii) the survival and infectivity of L1, that might be considered as important factors in establishing future goat infection, and iii) modifications induced with anthelmintic treatment on the above parameters.

* C.N.E.V.A., Station régionale de Pathologie caprine, 60 rue Pied de Fond, B.P. 3081, 79012 Niort Cédex, France.
** Correspondence author : INRA, Station de Pathologie aviaire et de Parasitologie, Unité d'Ecologie des Parasites, 37380 Nouzilly, France.

MATERIALS AND METHODS

FARMS AND FAECAL SAMPLINGS

Seven dairy-goat farms were investigated, from February to June 1991. They utilized permanent pasture throughout the year and were located in Centerwest of France (Deux-Sèvres). The mean temperature was 11.4 °C and annual rainfall ranged from 940 to 1327 mm. The size of flocks ranged from 35 to 180 goats. Individual faecal sampling (7 to 10g) were performed on 30 to 40 goats per farm when anthelmintic treatments had not been done for more than two months. A first sampling at day 0 (D_0) was done at the same time as an anthelmintic treatment (Rintal , Bayer Pharma, Puteaux, F : febantel, 5 mg/kg bodyweight). A second faecal sampling was done 21 days later (D_{21}). Malacological investigation was performed in November : all snails collected for 15 mn by 3 collectors were identified and the three most represented species are shown in Table I. The efficacy of febantel, initial LPG (larvae per gramme of faeces) and recent history of treatments are also shown.

Farm	Date of sampling	LPG	Number of anthelmintic treatments	LPG reduction on day 21 %	Intermediate host fauna (%)		
					Helix aspersa	*Deroceras reticulatum*	*Cernuella* sp
1	20/02	201	3 (0)*	69	5.8	38.2	29.9
2	18/03	147	5 (5)	32	0	100	0
3	20/03	182	5 (5)	23	21	33.9	0
4	15/04	105	3 (2)	30	33.8	36.5	0
5	17/04	294	2 (2)	51	6.6	54.7	0
6	12/06	158	6 (4)	34	28	5.4	58.1
7	25/06	140	2 (2)	54	0	86.2	0

* in brackets : benzimidazole compounds

Tableau I. – Parasitological characteristics of 7 dairy-goat farms : larval excretion in faeces at day 0 (LPG), susceptibility to febantel treatment, number of anthelmintic treatments per year, main intermediate host snails on pasture.

First stage larvae collection and preservation

The L1 were extracted from each sampled host by a modified Baermann technique (Cabaret *et al.*, 1980). The obtained larvae were pooled for each farm. The length of L1 was measured by means of a semi-automatic image analysis device Vids IV (Sirehill Industrial Estate, Saffron Walden, UK) on at least 30 larvae per farm and per period of sampling (D_0 and D_{21}). The remainder of larvae was stored at -20 °C in tap water for 4 to 8 months before use for snail infection.

Survival of L1 at various temperatures

For each farm six duplicated 20 g samples were respectively maintained under the following temperatures: 56 °C (2h or 3h), 20 °C (1 month), 4-6 °C (2 or 4 months), -20 °C during 4 months. Humidity of faeces was also modified under these conditions; the relative water loss was respectively 37.1, 48.4, 68.7, 67.4, 64.7, and 36.1%. The temperatures were chosen as to mimic normal to harsh conditions in winter (4-6°C and -20°C) or summer (20°C and 56°C). The L1 were extracted as described above ; survival was estimated as : (number of L1 at the collection time - number of L1 after test period)/ number of L1 at the collection time.

Infectivity of L1 : infection of *Helix aspersa*

The L1 of each farm preserved at -20°C were thawed and used to infect 9 to 16 *H. aspersa* laboratory reared and aged approximately of one month. The larval suspension used for infection contained 2L1 / µl and 30 L1 were in contact with each snail for 3 hours; snails were thereafter put together and maintained in one Petri dish per tested farm. Snails were examined 3 weeks post-infection for larvae; the shell was removed and the body directly examined for larvae at magnification 25.

Processing of data

A Stat-Itcf computer package (Manuel d'utilisation. Institut Technique des Céréales et des fourrages, 1988, Paris) was used to perform analysis of variance (Anova), principal component, correspondence and segmentation analyses.

RESULTS

Length of L1 in relation to farm origin and febantel treatment of goats

The average length on D_0 was 283.9 µm and standard-deviation 14.3 (255 L1). The distribution was normal: Pearson's coefficient of symmetry and kurtosis was respectively 0.003 and 2.5.
No significant difference was shown between farms and before/after treatment with febantel by Anova (respectively P=0.32 and P=0.31). However segmentation analysis on length of L1 on D_0 in relation to farm did show that two groups of L1 could be considered (P <0.03): less and over 280 µm, and that these two groups were differently represented between farms (Table II).

Farm	Percentage of L1	
	< 280 µ	> 280 µ
1	12	16
2	2	23
3	19	11
4	9	12
5	24	8
6	13	15
7	21	15
All farms	100 %	100 %

Tableau II. – Distribution of *Muellerius capillaris* first-stage larvae length in relation to farm origin

A higher frequency of small larvae (<280 mμ) was recorded in farms 3 and 5 and to a lesser extent in farm 7. Large larvae (>280 mμ) were predominant in farm 2.

L1 SURVIVAL IN FAECES AT 4 TEMPERATURES IN RELATION TO FARM ORIGIN AND FEBANTEL TREATMENT OF GOATS

The L1 survival was tested on D_0 and D_{21} after treatment. Significant differences (Anova) between test ($P < 0.001$) and between farms ($P < 0.01$) were found in survival values assessed on larvae sampled at D_0 and D_{21} (Table III). The variables farm and test were significantly interacting ($P < 0.01$) i.e. the L1 survival at one particular temperature was different from one farm to another ; correspondence analysis was thus appropriate to characterize L1 survivals in the 7 farms (Fig. 1). The analysed data matrix was as follows : the columns corresponded to ecological tests (survivals at 56, 20, 4-6 and -20°C) and the rows to the corresponding L1 survivals at D_0 ; the survivals at D_{21} were introduced as supplementary data i.e. they did not participate in the construction of correspondence analysis but were only located afterward on the plane determined by axes 1 and 2. At D_0 four groups (I to IV) of farms were distinguished :

* at 20°C and -20°C : i) farms 1 and 2 exhibiting high L1 survivals,
  ii) farms 4 and 5 with medium L1 survivals,
  iii) farms 3 and 6 with low L1 survivals,
* at all conditions : iv) farm 7 with low L1 survivals.

After anthelmintic treatment, the estimations of L1 survivals did not differ as much as before treatment; this was mostly due to a reduction of survivals at 20°C in farms 2, 4 and 5. The L1 survivals at 20°C remained high (32.8%) in farm 1.

L1 INFECTIVITY IN RELATION TO FARM ORIGIN AND FEBANTEL TREATMENT OF GOATS (Table IV)

No between farm ($P = 0.88$) or before/after febantel treatment ($P = 0.23$) significant difference in infectivity was recorded. The infection of *H. aspersa* was higher (1.3 *versus* 0.2-0.3) when fresh larvae were used (data not shown).

L1 VARIABILITY IN LENGTH, SURVIVALS AND INFECTIVITY IN RELATION TO FARM CHARACTERISTICS

A principal component analysis was performed on length (% under 280 μm), survivals at 56, 20, -20°C at D_0 and infectivity on D_{21} post-treatment (matrix columns were farms and rows were the respective values of the above mentioned parameters). Two clear groups, farm 1 and 2 (very good L1 survival at -20°C), and farms 4 and 5 (good survival at - 20°C and best infectivity scores) were found ; an intermediate group was constituted of farms 3 and 6, and farm 7 remained isolated (Fig. 2a). A similar analysis was performed on farm characteristics (see Table 1) : no obvious grouping was found (Fig. 2b). Farm characteristics were not apparently related to L1 variability as farm grouping was different between the two analyses presented in fig. 2.

Farm	56°C*		20°C		4-6°C*		- 20°C	
	D_0***	D_{21}	D_0	D_{21}	D_0	D_{21}	D_0	D_{21}
1	54.7	59.9	47.8	32.8	0.5	0	100	43.3
2	56.1	42.6	46.3	0.9	0.5	0.4	62.6	45.6
3	68.7	69.1	2.2	1	1.1	1.2	21.4	39.2
4	53.8	52.6	24.8	0.9	1	0	40	51.7
5	69.4	15.9	29.3	1	2.2	0.5	21.1	14.8
6	29.6	16.5	2.4	0.01	1.2	1.6	21	11
7	5.4	37.3	0.5	0.8	0.7	3.2	12.8	60.3
Mean	48.2	42.0	21.9	5.3	1.0	1.0	39.8	38.0
Coeff. of variation	47.7	48.8	94.2	226.6	56.8	115.4	78.8	48.5

* Average value (2 and 3 hours)
** Average value (2 and 4 months)
*** Days after treatment with febantel

Tableau III. – Mean survival rates of *M. capillaris* first-stage larvae in relation to farm origin and ecological test before (D_0) and after (D_{21}) febantel treatment of goats in seven dairy goat farms.

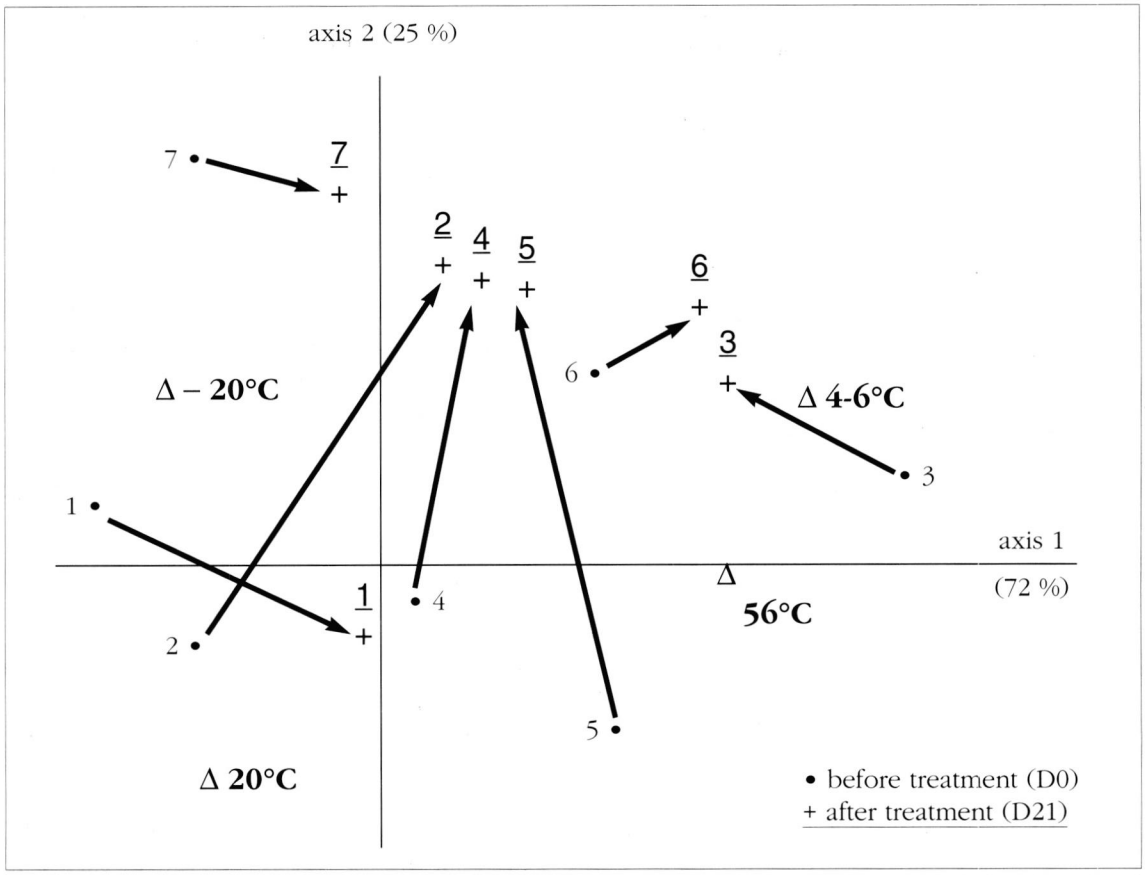

Fig. 1. – Between farms variations in *M. capillaris* first-stage larvae survival in faeces at 4 temperatures before (Day 0) and after (Day 21) febantel treatment of dairy-goats.Correspondence Analysis using farms as variables.

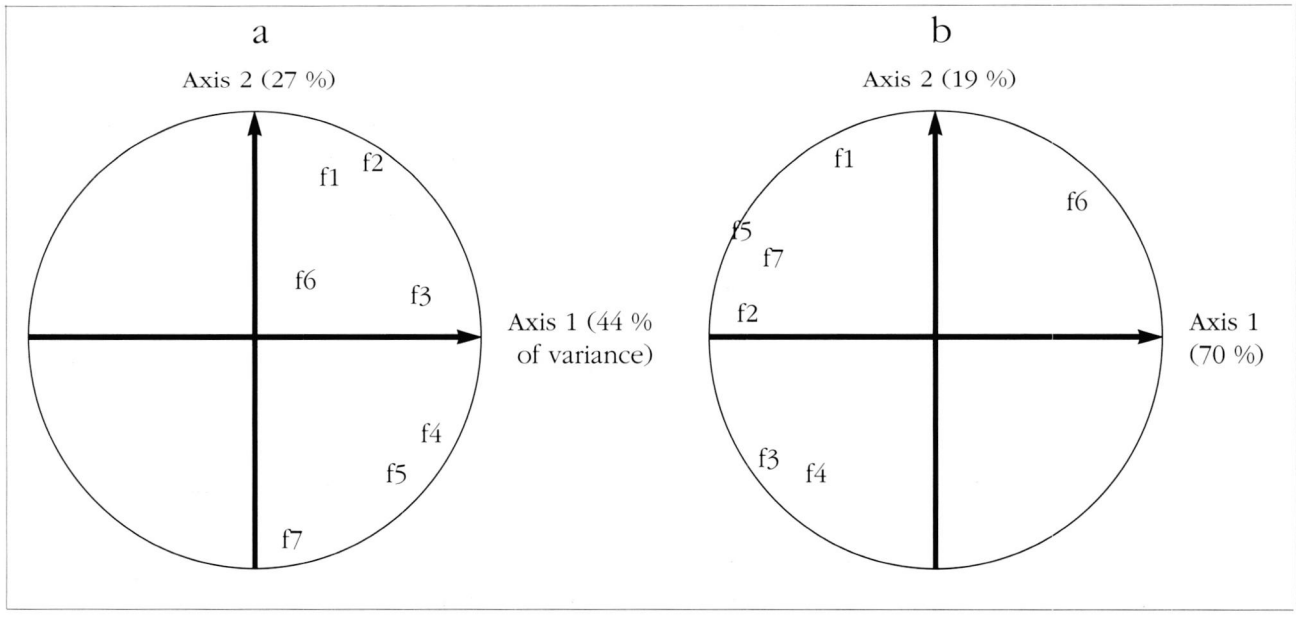

Fig. 2. – Characterization of farms by Principal Component Analysis :
similarities between farms in relation to *M. capillaris* first-stage larvae length, survival and infectivity (a) ;
similarities between farms in relation to L1 output in faeces of goats, efficacy of febantel treatment and intermediate host fauna (b)

Farm	Days after tratment with febantel	
	D0	D21
1	0.3* [0-1] (10)	0.4 [0-3] (14)
2	0.2 [0-1] (13)	0.3 [0-2] (12)
3	0.3 [0-2] (9)	0.3 [0-2] (12)
4	0.3 [0-2] (11)	1.1 [0-6] (9)
5	0.2 [0-1] (15)	1.1 [0-4] (15)
6	0.3 [0-2] (15)	0.1 [0-1] (14)
7	0.3 [0-2] (16)	0.4 [0-2] (12)

* Average number of second or third stage larvae/snail
[ ] Range
( ) N° of snails

Tableau IV. – Infection of the land-snail *Helix aspersa* with *M. capillaris* larvae obtained before (D_0) and after (D_{21})febantel treatment of goats in seven dairy-goat farm.

DISCUSSION

The between farms differences were recorded for L1 size and their survival capabilities at various temperatures, mostly 20 and -20°C. Between dairy-goat farms differences in ecology of trichostrongyle third-stage larvae has been also found in center of France (Gasnier and Cabaret, unpublished results), indicating that each farm has its own adaptated population. No obvious relation between the two parameters, size and survival of L1, could be found. The ability to infect an intermediate host was not significantly different from one farm to another (Anova) but principal component analysis suggested that the L1 from two farms might be more infective to the intermediate hosts. The only clear discriminating parameter is thus survival at cold or temperate temperatures in faeces.

The between farms differences were not related to parasitological characteristics of the farm (susceptibility to febantel treatment and intermediate-host fauna). Thus larval output was strongly reduced in farm 7, survival of L1 was poor at all temperatures tested, and L1 infectivity was average. It was as if the different management and environment between farms corresponded to various responses in susceptibility to treatment, larval survivals, but finally ended to similar larval infectivity. The adaptative significance of parasite susceptibility to treatment and larval ability to surviving in different conditions remained unclear.

The average L1 survivals were not much altered after treatment, but the between farm variability was reduced. This feature was striking and we may hypothesise that the phenomenon is of short duration as the reduction in L1 output after treatment corresponded probably to a temporary depletion of female worm fertility rather than to a destruction of adult worms (Dakkak, Cabaret, Ouhelli, 1979). We should reject the possibility of founder effects (establishment of very few worms in a flock) associated to genetic drift (random variation due to the small size of population). We could better search explanations in the modifications of environment of L1, for example duration of transit from lung to faeces and humidity of faecal pellets when emitted.

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