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## THE USE OF TRACER LAMBS FOR MONITORING PROTOSTRONGYLID INFECTION ON EXTENSIVE PASTURES OF MORROCO

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### Résumé

UTILISATION D'AGNEAUX TRACEURS POUR L'ÉTUDE DE L'INFESTATION PAR LES PROTOSTRONGYLES D'OVINS ÉLEVÉS SUR DES PÂTURAGES EXTENSIFS AU MAROC. — Trois agneaux traceurs ont été mensuellement mis sur un pâturage infecté par *Muellerius capillaris*, de décembre 1979 à septembre 1980. La densité et l'infestation de la malacofaune ont été enregistrées. Les lésions sur les poumons et la population parasitaire des poumons, des ganglions jéjunaux, médiastinaux et bronchiaux ont été examinés. L'infestation des limaces était maximale en février-mars, et celle des ovins en avril-mai. Le cycle interne est caractérisé par la longue durée de la présence des larves du troisième stade dans les ganglions lymphatiques. L'infestation des ovins semble principalement due à l'ingestion de larves libérées sur l'herbe après la mort des limaces.

The rate of infection by Protostrongylids is usually estimated either from the larval output in faeces or from the intensity of the lesions in slaughter material. The first evaluation is not always accurate even though the number of first-stage larvae (L1) has been shown to be related to the number of adults harboured in lungs (Cabaret *et al.*, 1980a) in one experiment. On the other hand, Rose (1959) was not able to correlate the output of larvae with the worm burden in lambs and a peri-parturient rise would modify the larval excretion (Cabaret, 1982). The value of the second evaluation is reduced by the variability of the animal samples.

The use of tracer lambs has been mainly restricted to monitoring gastro-intestinal nematode infections and has not been applied to assessing Protostrongylid infection. This could be due either

to the underating of the prevalence of the disease or to the difficulty of extracting the worms from the lung parenchyma.

Another method for evaluating periods at risk is to determine the infectivity of pasture (density of snails  $\times$  average infection per snail). One problem associated with this approach is the determination of the time-lag between pasture infection and actual infection of sheep. It must be remembered that the latter will depend on previous experience to the parasite (Ozerskaia, 1960; Denev, 1971), the level of larval intake and its time-distribution (Svarc, 1973) and very likely on the forage quality (Joyeux and Baer, 1951).

The aim of the present work is to monitor the infection of tracer sheep and to relate it to the pasture infectivity.

Table 1. — Protostrongylid infection in tracer lambs in Ain Aouda (Morocco)

Months	Lungs			Lymph nodes		
	Adults	L3 and L4	L1	Jejunal (L3)	Bronchial and Mediastinal	Faeces (L1/g)
D	0	1 (0-3) <sup>a</sup>	0	0.33 (1-23)	5 (0-8)	2 (0-6)
J	0.33 (0-1)	1 (0-2)	0	1.30 (1-2)	0	0
F	2.67 (2-6)	0-67 (0-2)	0	0.67 (0-2)	7.67 (3-16)	0
M	0	9.00 (7-11)	0	1.67 (0-4)	13.33 (7-22)	0
A	8.67 (1-19)	2.67 (1-6)	4553 (0-8580)	0.33 (0-1)	2.33 (0-6)	5 (0-7)
M	2.33 (1-3)	2.00 (0-5)	1176 (4-2773)	0.67 (0-1)	0.33 (0-1)	7 (4-12)
J	0	0.67 (0-2)	0	0	0	0
J	0	0.33 (0-1)	0	0	0	0
A	0	0	0	0	0	0
S	0	0	0	0	0	0

a: range

### Materials and Methods

The study was conducted on a farm of 1 000 ha located at 30 km from Rabat (Ain Aouda). The climate of the region is sub-humid with mild winters; the dry-season extends from mid-May to mid-October. Six flocks of each 150 ewes were grazed together on the pasture. Previous investigations (Pandey *et al.*, 1981) indicated that lungworm infection was monospecific (*Muellerius capillaris* Müller 1889); and that the intermediary hosts were slugs (*Milax gagates* Draparnaud 1801 and *Limax maximus* L. 1758).

#### — Tracer lambs

These were castrated lambs of local breed with BB haemoglobin type and their age ranged from eight to 14 months. Lambs were housed indoors one month prior to introduction to the natural flock. They were vaccinated against enterotoxaemia and treated with fenbendazole at 15 mg/kg (Dakkak *et al.*, 1979) even though apparently uninfected by Protostrongylids. The tracer lambs were then grazed on the pastures for one month.

At the end of this grazing period, they were again housed indoors for one month and then slaughtered. Three lambs were thus introduced monthly from December 1979 to September 1980.

#### — The intermediate hosts

Slugs were collected monthly from the same locations: near a temporary pond for *M. gagates* and in the tufts of *Iris sisyrynchium* L. for *L. maximus*. Owing to the size of the pasture, samples for the assessment of density (number of slugs/m<sup>2</sup>) were realized on another farm of the region of smaller size and higher mollusc density (170 samples areas of 0.66 m<sup>2</sup>). We obtained thus an index of density for the studied farm.

#### — Parasitological techniques

The following organs of sheep were examined: lungs and lymph nodes (20 g of jejunal, total bronchial and mediastinal). Lungs were cut into pieces (1 cm<sup>3</sup>) soaked in water for 7 h at 37 °C; the pieces were then removed and the liquid strained (mesh: 32 m). The larvae from lymph nodes were extracted after pepsic digestion (4 h,

Table 2. — Density and infection by *M. capillaris* of the slugs on the pasture in Ain Aouda (Morocco)

Months	Number of larvae/slug	p. cent of L3	Density (slugs/m <sup>2</sup> )
N	0.08 ± 0.08	0	0.03
D	0.50 ± 0.34	75	0.08
J	5.43 ± 3.56	84	0.30
F	6.42 ± 4.17	82	0.62
M	4.60 ± 3.06	83	0.03

41 °C). The number of larvae per gram of faeces was assessed using a technique described previously (Cabaret *et al.*, 1980b). The larvae (L1, L2 and L3) were extracted from slugs by pepsic digestion according to the procedure described by Cabaret (1979).

#### — Processing of data

The means are presented with their range or with a confidence interval (5%) established by the Rojas formula (Rojas, 1964), as the populations were not of a normal distribution.

## Results

### — Sheep infection

Three types of lung lesions were observed, according to the terminology of Beresford-Jones (1967): type A (petechial), type B (pseudo-tuberculous nodule) and type C (area of pulmonary densification). Type A was present through out all the period of study with a maximum in December; type B, though present during the whole period, was maximal in April-May. Type C was only observed in April-May.

The data concerning the parasitic populations are presented in table 1. The worm recoveries were low in lungs and lymph nodes. No larvae L3 was recovered from the caecal lymph nodes. First-stage larvae were shedded in faeces only in two occasions (April and May).

The results are presented in table 2. The infection rate was high from January to March. *M. gagates* appears in November as a juvenile (weight: 1.03 g); it becomes mature in January, lays eggs and dies progressively until the beginning of April. A similar behavior is recorded for *L. maximus*. Although the proportions of the two species were approximately equal, *M. gagates* was more infected than *L. maximus* (overall mean of  $5.60 \pm 1.35$  larvae per slug vs  $2.67 \pm 1.35$ ).

## Discussion

The studied farm presents a simple model as the only Protostrongylid is *M. capillaris* and the

only intermediate hosts are slugs. Usually the Protostrongylid fauna is multispecific (Joyeux and Gaud, 1946; Cabaret *et al.*, 1980c) and the malacofauna includes both snails and slugs (Cabaret *et al.*, 1980c).

### — Characteristics of sheep infection

The figures numbers of lungworm were low; this may be due either to the low susceptibility of lambs (Ramirez-Fernandez, 1967; Trushin, 1971; Cabaret *et al.*, 1978) low uptake of larvae or to the low rate of recovery of worms from the parenchyma by our technique. Perfusion techniques are available (Inderbitzin, 1976) and have been used with success for *Dictyocaulus sp.* but they are not very convenient for Protostrongylids.

The first-stage larvae (L3) were still in lymph nodes one month after the last infection, even though Boev (1975) and Castanon-Ordenez (1982) estimated that their maximal stay is of one week. L3 are usually recovered from caecal lymph nodes but we did not isolate larvae from these lymph nodes in our case: it may be due to the fact that Protostrongylid species were different. L3 were also recovered from lung lymph nodes and this has never been recorded. This is not restricted to lambs: we have extracted up to 300 L3 from lung lymph nodes from naturally infected ewes (unpublished). L3 were isolated from the lungs which suggests that no development had occurred of the ingested infective larvae. This is in agreement with the results of Castanon-Ordenez (1982) for *Neostrongylus linearis*, Azimov *et al.* (1975) for *M. capillaris* but at variance with those recorded by Boev (1975) and Svarc (1973). The presence of larvae in lymph nodes is explained by the normal route of migration. Some indication of the rate of migration can be obtained from the ratio; total parasites in lungs/larvae in lymph nodes. This index fluctuates from December to May as follows: 0.07 — 1.00 — 0.40 — 0.60 — 4.20 — 4.30 — and is at its maximum when the output of L1 (in lungs and/or in faeces) is high i.e. in April-May. High values are associated with type C lesions which is in agreement with the data of Beresford-Jones (1967). Similar variations in the

duration of the pre-patent period has been observed previously in experimental infections (Cabaret and Larson, 1981) and in slightly infected lambs (100-300 L3/lamb) it can extend for up to six months. Such variations in the duration of the prepatent period could possibly explain the results of Rose (1959) on *M. capillaris* where his observation lasted for less than two months and a significant percentage of lambs did not apparently become infected.

— *Characteristics of the infection of the intermediate hosts*

November is the beginning of the infection (table 2) when the population of naive slugs emerges from the ground. The highest level of infection (number of larvae per slug) occurs from January to March. The high output of L1 larvae from sheep at this period (Cabaret, 1981) and the increasing activity of slugs (mating, egg laying) are considered responsible for this rise in infection. Other results suggest that experimental susceptibility of *L. maximus* is at its highest in January-February when they are adult and simultaneous infection of juveniles and adults have shown that the latter are more susceptible (Cabaret, 1981). Another study in the Rabat area (Cabaret *et al.*, 1980c) indicated that the infection of slugs was maximal during the same period and that *M. gagates* was more infected than *M. maximus* (9.70 larvae per slug vs 0.75).

— *Infection of sheep and pasture infectivity*

The pasture infectivity is maximal in February and the infection of sheep in April. Surprisingly, in April few slugs are present; however, infection could occur without the presence of any slugs. One can hypothesize that the larval L3 migrate out

of the slugs into the grass, survive and are ingested when the lambs graze. Migration out of the foot of the dead intermediate host has been recorded by Martinez-Morales (1967), and Cabaret (1981) and there is evidence that they can survive for 90 days at 5-8 °C and 30 days at 30 °C (Martinez-Morales, 1967). The L3 of *M. capillaris* can also withstand sequences of dessication and are not easily destroyed by sunlight (Cabaret, 1981). Thus, the infection of sheep directly by L3 liberated after the death of molluscs is probable, as Davtian (1947) remarked. In natural conditions we were able to infect goats with *M. capillaris* which were grazing on a pasture previously contaminated with pieces of dead infected slugs (Cabaret, 1982). Owing to the time-lag, pasture infectivity would appear to be a good parameter for predicting the future level of infection of sheep.

In conclusion, we suggest that the epidemiology of *M. capillaris* in Moroccan conditions can be summarized as follows. From November through to March (i.e. during the rainy season) the slugs are active and become progressively infected. After their death (March-April), the infective larvae are liberated on the grass. Infection of sheep can occur throughout the rainy season, but is particularly important from March to May, when they ingest the liberated larvae. Infection is efficient at that time, as the prepatent period is shortened. The rate of migration seems to be more rapid in April-May and slower from December to March and thus the duration of the life-cycle seems to fluctuate. The factors influencing this duration are unknown; age of L3 or larval doses might interfere.

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## Summary

Three tracer lambs were grazed each month from December 1979 to September 1980 on pastures infected by *Muellerius capillaris*. At slaughter, the lesions on the lungs and the parasite population in lungs and jejunal, mediastinal and bronchial lymph nodes were examined. The density and infection of the malacofauna were also recorded. The infection of slugs reached a maximum in February-March, and that of sheep in April-May. The internal life-cycle was characterized by the long duration of third-stage larvae in lymph node. It is postulated that infection of sheep was mainly due to the ingestion of larvae liberated onto the grass after the death of slugs.

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