

DYNAMICS OF THE ELIMINATION OF *DICROCOELIUM DENDRITICUM* (TREMATODA, DIGENEA) EGGS IN THE FAECES OF LAMBS AND EWES IN THE PORMA BASIN (LEÓN, NW SPAIN)*

M. Y. MANGA-GONZALEZ, C. GONZALEZ-LANZA, P. DEL-POZO-CARNERO

SUMMARY

The elimination of *Dicrocoelium dendriticum* eggs in sheep faeces from 4 localities in the upper and middle Porma river basin was recorded at monthly intervals between March 1986 and March 1987. The faecal material was taken directly from the rectum of the animals (10 % of the flock in each village). The sedimentation technique and McMaster egg counting chambers were used to analyze the faecal samples. Of the 995 samples examined, 63.6 %

contained *D. dendriticum* eggs and the number of eggs per gram (epg) varied from 33 to 5,340 (average 323.4 ± 18.5). The highest elimination rate was observed in winter, not only because of prevalence (90.5 %) but also because of the epg (average 726.6 ± 88.4). Prevalence of infection (from 61.5 to 65.4 %) was similar for all age groups, although the highest mean epg value (367.6 ± 50.9) was found in lambs.

RÉSUMÉ : Dynamique de l'élimination d'œufs de *Dicrocoelium dendriticum* (Trematoda, Digenea) dans les fèces des ovins du Bassin de la Porma (León, Nord-Ouest de l'Espagne).

De mars 1986 et mars 1987, à intervalles mensuels, l'élimination d'œufs de *Dicrocoelium dendriticum* dans les fèces d'ovins a été mesurée dans 4 localités du bassin supérieur et central du fleuve Porma. Les matières fécales étaient prises directement au rectum des animaux (sur 10 % du troupeau d'ovins de chaque village). Les échantillons de fèces sont analysés selon la technique de sédimentation et les œufs comptés en cellule de McMaster. Des

995 échantillons examinés, 63,6 % contenaient des œufs *D. dendriticum* et le nombre d'œufs par gramme (opg) variait entre 33 et 5 340 (moyenne $323,4 \pm 18,5$). Le niveau d'élimination le plus haut (prévalence et opg) était rencontré pendant l'hiver. La prévalence d'infestation (de 61,5 à 65,4 %) était similaire pour tous les groupes d'âge, bien que le niveau moyen le plus haut d'opg ($367,6 \pm 50,9$) soit rencontré chez les agneaux.

INTRODUCTION

Dicrocoeliosis, a disease produced by the small liver fluke *Dicrocoelium dendriticum* (Rudolphi, 1819) Loos, 1899, is a common parasitic disease in ovine and bovine livestock in Europe, Asia and North America (Soulsby, 1968).

This Digenea uses different species of land molluscs as first intermediate hosts and various species of ants as second intermediate hosts in its complex biological cycle (Tarry, 1969; Jolinja *et al.*, 1972; Paraschivescu, 1981; Manga, 1983). Nevertheless, integrated studies on the referred parasitic disease are scant, probably due to the complexity of its life cycle (Boray, 1981).

The epidemiology of dicrocoeliosis depends largely on local environmental and ecological factors, as well as the ethology of their intermediate and definitive hosts. Thus, in general, the results obtained in other countries cannot be applied to ours.

D. dendriticum is widespread in the Iberian Peninsula, according to the survey published by Cordero *et al.* (1980). However, very few studies on the epidemiology of this disease have been carried out in our country and, in any case, they are partial contributions (Del Rio, 1967; Rojo *et al.*, 1981; Alunda, 1984; Manga, 1987, amongst others).

The aim of the present work is the assessment of faecal egg output in relation to season, locality and age of sheep in the Porma basin (León).

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Unidad Estructural de Parasitología Animal, Estación Agrícola Experimental, Consejo Superior de Investigaciones Científicas (CSIC), Aptdo. 788, 24080 León (Spain).

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MATERIALS AND METHODS

Between March 1986 and March 1987, the elimination of *D. dendriticum* eggs was observed at monthly intervals at 4 locations of the upper and middle Porma basin. The sampling places were:

Vegaquemada (U. T. M. coordinates: 30TUN0943; altitude: 936 m), Orones (30TUN1759; 1 200 m), Redipollos (30TUN1663; 1 150 m) and Cofiñal (30TUN1566; 1 118 m). These locations are situated in the León mountain region with a continental climate within the Mediterranean-Atlantic transition. According to the meteorological data from the two stations in the study area for the period 1951-1980, which were supplied by the staff of the Duero basin Meteorological Service (Valladolid, Spain), the mean monthly maximum temperature ranges between 14.8 and 32.6° C, the mean monthly minimum between - 14.8 and 0.5° C and the mean monthly average between 1.05 and 15.05° C. Average monthly precipitation also varies between 36.5 and 163.1 mm.

Ten per cent of the actual number of sheep (crossbred Merino and « Churra ») in each village was monthly sampled. In each location (except in Vegaquemada) the animals belonged to different owners (the number varied from 4 to 7), although the pastures were communal and all the animals from each village went out to graze together. Bearing in mind the above mentioned, all the animals from each village can be considered as one flock. Nevertheless, we must point out that animal management in stable may be a little different. The number of animals tested in each sampling varied due to incidences such as slaughtering, selling and new young animals being included. According to our information only the sheep from Vegaquemada received two anthelmintic treatments: one in April (Rafoxanide) and another in May (Fenbendazol), although we do not know the exact dose administered.

The animals sampled went out to pasture in the morning and returned to their stables in the evening throughout the year, except for some days in winter when the pastures were completely covered

with snow. The main lambing period extended from February to March, although some ewes lambed during autumn.

The sheep were divided into the following groups for the age influence study : 1) less than 1 year, 2) from 1 to 4 years and 3) over 4 years. The age was assessed from teeth observations.

The faeces were taken early in the morning from the rectum of animals. These were not tagged, so in each sampling the faeces were taken at random from animals belonging to each one of the sheep age groups established. Three grams of faeces of each animal were processed by the sedimentation method and McMaster chambers were used for the egg counts (expressed in eggs/gram of faeces: epg).

In order to discover differences in infection prevalence in relation to sampling localities, collection months and sheep age, the chi-square test (χ^2) was used. One-way analysis of variance and calculation of the lowest significant difference (LSD) between mean values were carried out to determine whether there were differences in the number of eggs per gram with regard to the localities from which the samples came, sampling month and age groups of the animals.

RESULTS

Of the 995 samples taken, 63.6 % contained *D. dendriticum* eggs. Epg varied between 33 and 5 340, with an average (\bar{x}) of 323.4 ± 18.5 (\pm Standard Error = SE).

As far as infection according to sampling localities was

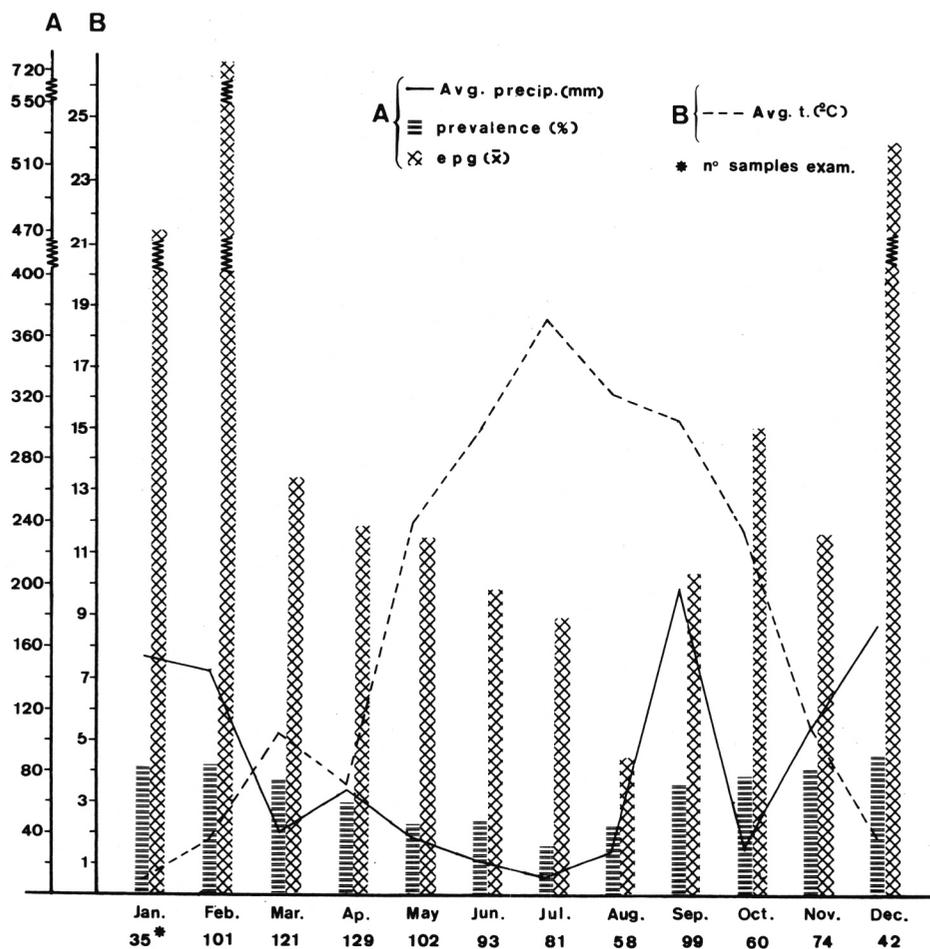


FIG. 1. — Monthly dynamics of *D. dendriticum* egg elimination. Temperature (t.) and precipitation (precip.) values corresponding to sampling period.

TABLE I. — Infection prevalence (%) and average number of *D. dendriticum* eggs per gram (epg) in sheep faeces at four locations in the Porma river basin.

Sampling location	Parasitological results	
	% infected (No. exam.)	Avg. epg \pm SE (range)
Vegaquemada	51.0 (273)	257.0 \pm 27.2 (33-2 000)
Orones	66.3 (273)	311.7 \pm 26.9 (33-3 267)
Redipollos	73.7 (235)	398.8 \pm 52.0 (33-5 340)
Cofiañal	65.0 (214)	310.6 \pm 28.8 (67-2 800)

concerned, maximum prevalence (73.7 %) and highest average epg (398.8 \pm 52) were recorded for Redipollos, whilst the minimum for both parameters was detected in Vegaquemada (51 % and 257 \pm 27.2, respectively) (table I). However, it must be pointed out that the animals from this last locality received two anthelmintic treatments, as it was previously mentioned.

As can be seen in figure 1, *D. dendriticum* eggs were found in sheep every month in the Porma region. The main period of egg elimination was detected at the end of autumn and in winter. Maximum level for prevalence (90.5 %) was detected in December and for average epg in February (726.6 \pm 88.4). The minimum figures were obtained in summer, that is, in July for prevalence (30.9 %) and in August for the average number of epg (90 \pm 9.7).

A seasonal dynamics pattern similar to the above-mentioned one was observed among all the different localities, except in Orones in which the maximum average epg was detected in May (550.3 \pm 392.2).

According to the total results summarized in figure 2, it seems that sheep age does not have a clear influence on egg elimination, due to the fact that prevalence (from 61.5 to 65.4 %) and average epg (from 297 \pm 16.8 to 367.6 \pm 50.9) were similar in the established groups. In spite of this, a slight increase in prevalence was observed from the first age group to the third one.

Nevertheless, prevalence and average epg differed at each of the sampling points in relation to animal age. Thus, we have observed from a decrease in both parameters with age in Orones (73.3 % and 331.3 \pm 54.3 epg \bar{x} in sheep less than 1 year old; 67.7 % and 308.3 \pm 59 epg \bar{x} in the oldest sheep), to the opposite situation, in relation to prevalence, in Vegaquemada (38 % and 55.6 %, respectively).

By means of the χ^2 test, statistically significant differences were observed among the sampling localities, with regard to infection prevalence ($\chi^2 = 31.4$; $P \leq 0.005$; d.f. = 3). On applying the 2 \times 2 contingency tables signi-

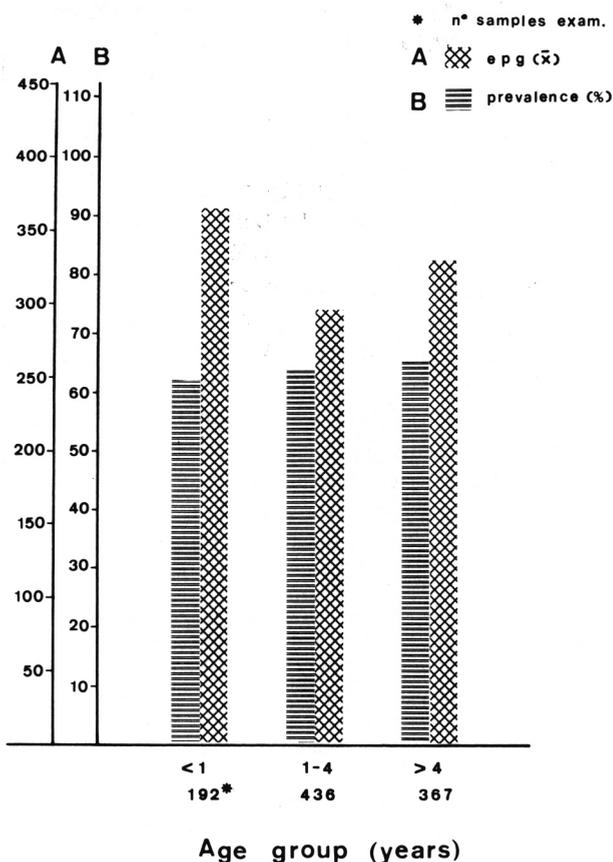


FIG. 2. — Rate of *D. dendriticum* egg elimination by sheep age groups.

ficant differences were discovered for $P \leq 0.05$ between Vegaquemada and the other localities and between Cofiañal and Redipollos.

Statistically significant differences were similarly detected among sampling months, for infection prevalence ($\chi^2 = 130.2$; $P \leq 0.005$; d.f. = 10). We must point out that November and December dates were processed together. By means of 2 \times 2 contingency tables, significant differences ($P \leq 0.05$) were observed between several months. Nevertheless, the highest χ^2 values were recorded between: 1) May and November-December, 2) July and February, 3) July and March, 4) July and November-December.

Using the χ^2 test, no statistically significant differences were recorded among the animal age groups with reference to prevalence.

When one-way analysis of variance was applied statistically significant differences ($P \leq 0.005$) were only noticed among the sampling months for the number of eggs per gram. When pairs of epg mean values were compared, by means of the LSD test, significant differences for $P \leq 0.05$ were observed between: 1) January and the rest of the months, except October and December; 2) February

and all the other months; 3) August and October; 4) December and the rest of the months.

DISCUSSION

The levels of prevalence found by us are some what lower than those obtained in Spain by Del Rio (1967) in ovine from the León mountain region (100 %) and by Garcia and Juste (1987) in the Basque country (100 %); in the district of Alpes-Maritimes of France (83.1 %) by Calamel (1976); in Italy (100 %) by Ambrosi and Principato (1981); in the Marmara region (Turkey) (64-74 %) by Kalkan (1971). However, the infection prevalence in sheep of the Porma region was higher than those obtained in the southeast of France by Calamel (1976) (44.9 %) and Brunet (1981, cited by Mangeon and Cabaret, 1987) (16-30 %) and by Lietava (1984) in Czechoslovakia (54.8 %).

The number of *D. dendriticum* epg found by us was considerably higher than that obtained by Rojo *et al.* (1981) in León (Spain) (13-773 epg). If we take into account the maximum rate of elimination, our results (5 340 epg) were also superior to those obtained by Kalkan (1971) (maximum 685).

As far as seasonal incidence is concerned, our findings do not agree with those of Kopp (1975) who found the highest elimination of eggs in spring (March to May). Here our results coincide only partially with those of Garcia and Juste (1985) and Ambrosi and Principato (1981), because they obtained the egg elimination maxima in spring and autumn and from February to May, respectively.

According to the authors consulted by us the number of *D. dendriticum* eggs eliminated in the faeces increases with sheep age (Kopp, 1975) as well as the level of flukes (Baker, 1950; Denev *et al.*, 1970). Nevertheless, when our results are taken into account sheep age does not seem to have a clear influence on egg elimination. If the animal owners gave us all the information referring to anthelmintic treatments, then we do not have a concrete explanation for this disagreement with the observations of other authors; perhaps our results could be affected by the much lower number of younger animals examined.

When the correlation established by Calamel and Giauffret (1976) between the coprological and parasitic burden results was considered, the infection level of the flour flocks studied by us fell into the category called confiscation-infestation (+ + +) by them. Nevertheless, according to our coprological results and taking into account the relation established by Rojo *et al.* (1981) between the faecal elimination and the parasitic burden, it could be gathered that one of the flocks examined by us harboured a parasitic burden between 5 000 and 10 000 flukes and the others between 3 000 and 10 000.

According to dynamics infection of the first intermediate hosts (Manga, 1987), the observation time of the slime-

balls with cercariae (Del Rio, 1967) and the period in which ants harbouring metacercariae have been observed in the tetanic stage (our unpublished data), we think that most of the young molluscs become infected between the beginning of spring and the beginning of summer. Some of these snails survive as adults until the next year. These adult molluscs would expel the mature cercariae, inside the slimeballs, principally during the spring until the beginning of summer. The cercariae eaten by the ants become infective metacercariae for the definitive hosts from the end of spring until the beginning of autumn. According to this, *D. dendriticum* egg elimination in the faeces of the definitive hosts (infected by eating ants which harbour infective metacercariae) would start at the end of autumn and continue throughout the winter. This hypothetical situation seems to be confirmed in as far as the ovine definitive host egg output is higher at the end of autumn and winter.

Generally speaking, our previously described dicrocoeliosis pattern coincides in part with that given out by Badie (1978) for the region of Limousin (France), who considered that the infection period for the ovine and cattle animals took place from May to October and clinical manifestations of the dicrocoeliosis in sheep appeared very often in autumn and in winter.

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