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Coprological study of the *Dicrocoelium dendriticum* (Digenea) egg elimination by cattle in highland areas in León Province, Northwest Spain

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Abstract. *Dicrocoelium dendriticum* egg output in cattle from five locations of the Porma river basin (León Province, Northwest Spain) was studied at monthly intervals between March 1986 and March 1987. We found *D. dendriticum* eggs in 37.64% of the 1251 samples examined, and the number of eggs per gram (epg) ranged from 10 to 1000 (average, 41.65 ± 2.73). The main egg-elimination period, for prevalence, was autumn-winter. The average epg values were quite similar during all months except March, when the maximum was detected. The infection prevalence increased in cattle aged up to 2 years and then a slight, gradual decrease was observed with increasing host age. The average epg values ranged from 40.08 ± 3.24 to 48.33 ± 12.42 in all age groups except the oldest animals, for which the figures were lower. The rate of egg elimination was higher in animals that came from locations situated at a greater altitude.

Dicrocoeliosis is a helminth infection distributed worldwide and, in our country, is produced by the small liver fluke *Dicrocoelium dendriticum* (Rudolphi 1819) Loos 1899. The complex life cycle of the fluke involves several species of land molluscs as first intermediate hosts, various species of ants as second intermediate hosts and a wide range of mammals, generally ruminants, as definitive hosts. The importance of this parasitic disease from the point of view of economy and health has been mentioned by Panasyuk et al. (1972) and Boch and Supperer (1982). The epidemiology of dicroceliosis depends not only on the behaviour of the parasite as well as the ethology of its hosts, but also on the local environmental and ecological factors. Therefore, the conclusions reached in other countries on this matter (Mapes 1951; Tarry 1969; Kalkan 1971; Jolinja et al. 1972; Badie 1978; Ben Amer and Ahmed 1980; Paraschivescu 1981; Bocharova 1984) are not valid for Spain.

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According to the survey carried out by Cordero et al. (1980), dicrocoeliosis is very widespread in the Iberian peninsula. Despite the partial contributions about the prevalence of infection by *D. dendriticum* in livestock (Del-Rio 1967; Mañas-Almendros et al. 1978; Rojo-Vázquez et al. 1981; Manga-González et al. 1991a) and in the first and second intermediate hosts (Del-Rio 1967; Manga-González 1983, 1987; Alunda 1984; Alunda and Rojo-Vázquez 1984; Manga-González et al. 1991b), the knowledge about the parasite's transmission is scant. To contribute to a better understanding of *D. dendriticum* transmission in our region, we decided to follow the egg elimination of this parasite in relation to the season, the locality and the age of cattle in the Porma basin (León, Northwest Spain).

Materials and methods

Between March 1986 and March 1987, *Dicrocoelium dendriticum* egg elimination was recorded at monthly intervals at five locations in the upper and middle Porma basin: Vegaquemada (U.T.M. coordinates: 30TUN0943, altitude 936 m), Primajas (30TUN2155, 1100 m), Orones (30TUN1759, 1200 m), Redipollos (30TUN1663, 1150 m) and Cofiñal (30TUN1566, 1118 m). The localities are situated in the León transition and mountain regions with a continental climate within the Mediterranean -Atlantic transition. According to the meteorological data reported from the two stations in the study area for the period 1951–1980, which were suppplied by the staff of the Duero basin Meteorological Service (Valladolid, Spain), the mean monthly maximum for 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. The average monthly precipitation varies between 36.5 and 163.1 mm.

In each location, 10% of the cattle (Brown-Alpine) were sampled at monthly intervals. The farming system was of the extensive type, i.e. the animals went out to pasture in the morning and returned to their sheds at night except during winter, when they were kept in sheds. The cattle normally grazed at the bottoms of valleys, in hay-meadows that belong to the *Molinio-Arrhenatheretea* R.TX., 1937 communities, although they also grazed on dry pastures of *Festuco-Brometea* BR.-BL./R.TX., 1943 and moister communities belonging to *Nardo-Callunetea* PRSG., 1949.

For the age-influence study the cattle were divided into the following groups: group 1, <1 year; group 2, 1 year; group 3, 2 years; group 4, 3 and 4 years; group 5, 5 and 6 years; group 6, 7 and 8 years; group 7, 9 and 10 years; and group 8, >10 years. Faeces were collected early in the morning directly from the rectum of animals chosen at random from each of the age groups established. In all, 10 g faeces from each animal was processed by the sedimentation method and McMaster chambers were used for the egg counts (expressed in eggs per gram of faeces, epg). To discover differences in infection prevalence in relation to sampling localities, collection months and cattle age, the chi-square test 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, the sampling month and the age groups of the animals.

Results

Dicrocoelium dendriticum eggs were found in 37.64% of the 1251 faecal samples studied. The epg values ranged between 10 and 1000, with the average (±SE) being 41.65±2.73. As can be seen in Table 1, the highest percentage of animals eliminating eggs was recorded in Cofiñal (50.23%), although the highest average epg value (48.39±8.98) was found in Redipollos. The lowest value for both parameters was detected in Vegaquemada (22.37% and 30.20±3.34, respectively).

D. dendriticum eggs were found in bovine faeces every month in the Porma region. Generally speaking, the main egg-elimination period (Fig. 1) in terms of prevalence was autumn-winter, with the maximal value being recorded in January (59.74%). The monthly average epg values oscillated between 26.08±2.86 and 41.17±6.37, except in March, when this value was higher (100.39±22.85). When data obtained at the different sampling points and months were taken into account, a pattern similar to that mentioned above was observed, with maximal prevalence values being recorded from September (Primajas) to January (Cofiñal). There was no uniformity in the average epg results obtained, since the maximal value was recorded in spring for three localities and in autumn-winter for the rest.

The infection prevalence increased with cattle age up to group 3 (2 years), when the maximum was recorded (47.87%, Fig. 2). From this point onwards a slight, gradual decrease was observed with increasing host age. The average epg values ranged from 40.08±3.24 to 48.33±12.42 in all groups except the animals older than 9 years, with the average value being 30. On examination of the influence of age at each of the sampling points on prevalence, the maximal values were observed in cows between 2 and 4 years old for all localities except Vegaquemada, where the highest prevalence was found in animals aged 7 and 8 years. In terms of the epg values obtained in the various age groups, the situation varied irregularly not only at each location but also between the five locations.

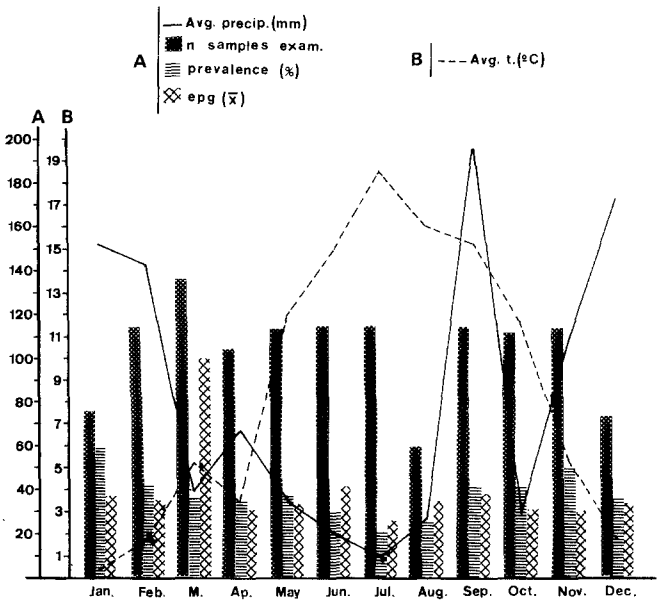

Fig. 1A, B. Monthly dynamics of *Dicrocoelium dendriticum* egg elimination, showing **A** average (*Avg.*) precipitation (*precip.*) and **B** average temperature (*t*) values corresponding to the sampling period

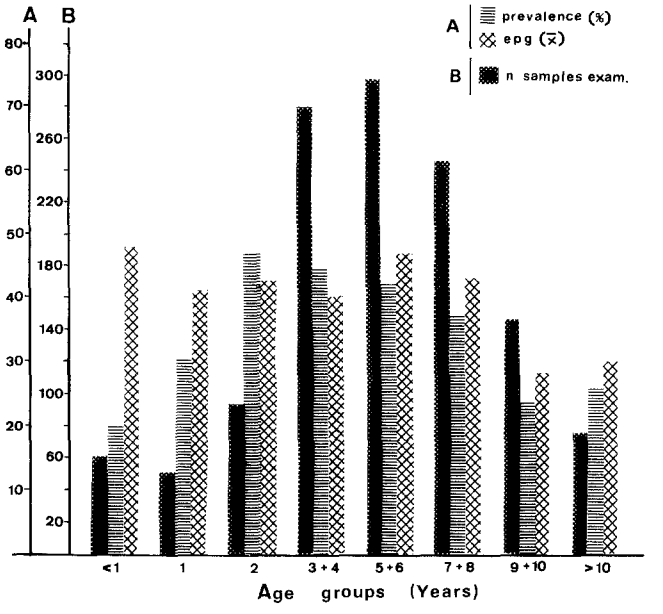

Fig. 2A, B. Rate of *D. dendriticum* egg elimination by cattle according to age group

Table 1. Infection prevalence and average number of *Dicrocoelium dendriticum* eggs detected in cattle faeces at five locations

Sampling location	Samples		epg		
	Number examined	% Infected	X̄±SE	Maximum	Minimum
Vegaquemada	219	22.37	30.20±3.34	160	10
Primajas	150	32.00	33.67±3.01	120	10
Orones	65	36.92	39.56±7.91	180	10
Redipollos	393	34.86	48.39±8.98	1000	10
Cofiñal	424	50.23	42.01±2.21	180	10

When the chi-square test was applied, statistically significant differences in infection prevalence were noted among the months in which the samples were collected ($\chi^2 = 47.92$; $P \leq 0.005$; $df = 11$). On application of the 2×2 contingency tables, significant differences were discovered for $P \leq 0.05$ between several months. Nevertheless, the highest χ^2 values for $P \leq 0.005$, shown in decreasing order, were recorded between: (1) January and July, (2) July and November, (3) January and June and (4) January and August. Similarly, for prevalence of infection, statistically significant differences were detected among the age groups ($\chi^2 = 34.59$; $P \leq 0.005$; $df = 7$). By means of 2×2 contingency tables, significant differences were discovered for $P \leq 0.005$ between the following groups according to animal age: group 1 and groups 3–5; group 7 and groups 3–5; and group 8 and groups 3 and 4. The level of significance was lower between groups 1 and 6; groups 2 and 3; groups 5 and 8; and groups 6 and 7.

Statistically significant differences were also recorded with regard to infection prevalence when the sampling localities were taken into consideration ($\chi^2 = 53.73$; $P \leq 0.005$; $df = 4$). On application of the 2×2 contingency tables, significant differences were observed for $P \leq 0.05$ between Cofiñal and the other localities and between Vegaquemada and all other sampling points.

By means of one-way analysis of variance, statistically significant differences were observed only in relation to epg values among the sampling months ($F = 5.26$; $P \leq 0.005$; $df = 11$ and 460). When pairs of the mean egg-count values were compared taking into account the lowest significant differences (LSD) test, significant differences for $P \leq 0.05$ were detected only between March and the rest of the months.

Discussion

Our findings on infection prevalence were more or less similar to those recorded for cattle in Spain by García and Juste (1987) in the Basque Country (35.71%) and by Mañas-Almendros et al. (1978) in Granada province (34.38%; we must point out that this value refers to eggs from the gallbladder) and in Azerbaidzhan (30%) by Sadykhov and Melikov (1980). However, the level of prevalence found by us was higher than those observed in Spain by Reina et al. (1987) in Cáceres province (0.7%), in Italy by Batelli et al. (1987) in Florence (5%), in Bulgaria (0.5%-21%) by Denev et al. (1970) and in Pakistan (1.51%) by Afzal et al. (1981). Our recorded prevalence was also higher than the data reported in slaughterhouse studies carried out in Sweden (0.8%–26%) by Bengtsson et al. (1968), in Libia (27%–35%) by Ben Amer and Ahmed (1980), in Russia (2.7%) by Bausov et al. (1981) and in Italy (6.8%) by Ambrosi et al. (1982). In contrast, the rate of infection found by us was obviously lower than that obtained by Del-Rio (1967) in the same region in coprological studies as well as in liver research (97.9%–100%). Likewise, Cagnolati and Ambrosi (1985) detected a higher infection prevalence (60.6%).

The *Dicrocoelium dendriticum* epg value found by us was considerably greater than that obtained by García and Juste (1987) in the Basque County (Spain) in two surveys, one carried out only in October and the other, over a 1-year period. The main egg-elimination period in terms of prevalence was recorded in autumn-winter, and this finding coincides partially with the results reported by Mañas-Almendros et al. (1978) and Ambrosi et al. (1982), who detected the highest rate of infection in autumn. However, our findings on the seasonal variation of epg values do not agree with those of Kopp (1975), who found the highest elimination of eggs in autumn, or that of García and Juste (1987), who observed egg-elimination maxima from April to May and June to July.

According to our results, cattle age does not seem to have a clear influence on egg elimination, although most of the previous authors pointed out that the infection by *D. dendriticum* was higher in older cattle (Bengtsson et al. 1968; Mañas-Almendros et al. 1978; Cagnolati and Ambrosi 1985; Rojo-Vázquez 1985). However, we found maxima for prevalence in 2-year-old hosts. We do not have a concrete explanation for this disagreement, but our results may have been affected by the lower number of younger animals examined. The data obtained in this work seem to confirm the general pattern of *D. dendriticum* transmission in the province of León described by Manga-González et al. (1991a).

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