

Dicrocoelium dendriticum primoinfection and kinetic egg elimination in marked lambs and ewes from the León mountains (Spain).

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In Memoriam

Dedicated to the memory of Prof. Dr. Ignacio Navarrete, who was always a caring friend from our first meeting in Cambridge in 1980. I will never forget his kind words to me during the homage to Prof. Cordero in 1992, or the beautiful plants that he and our colleagues from Cáceres and Cordoba sent me. We will always remember his happiness and good humour, as well as his intensive work and conciliatory aptitude in the Spanish Society for Parasitology.

Abstract: In order to establish the primoinfection period and monitor the *Dicrocoelium dendriticum* egg elimination kinetic in marked ovines grazing in the mountains of León (NW, Spain), faeces samples were collected monthly for two years from the rectum of 81 animals aged between four months and eight years at the start of the experiment. They were analyzed (by sedimentation and McMaster). Some lambs started to shed eggs two months after their first outing, although most did so after four months. All the animals studied shed parasite eggs with the faeces, either in all of the samples or some of them, and the eggs per gram (epg) number oscillated between 67-2533 ($\bar{x}$ 244.50 $\pm$ 8.10 SE). The highest percentage of animals eliminating eggs (86.66%) and the highest value of the mean epg (374.61 $\pm$ 38.5) were detected in February. In the oldest animal group (those aged 7-8 years) the highest values of the positive sample percentage (82.86%) and the epg mean (372.60) were detected, while the lowest figures were detected in the lambs (aged 4-8 months).

Key words: *Dicrocoelium dendriticum*, marked ovine, natural infection, egg elimination kinetic, León, Spain.

Resumen: Para establecer la época de primoinfección y seguir la cinética de eliminación de huevos de *Dicrocoelium dendriticum* en las heces de ganado ovino marcado y mantenido en pastoreo en la montaña de León (NW, Spain), se recogieron y analizaron (por sedimentación y McMaster) muestras de heces tomadas mensualmente, durante dos años, del recto de 81 animales, con edades comprendidas, al principio del experimento, entre cuatro meses y ocho años. Algunos corderos iniciaron la eliminación de huevos a los dos meses de su primera salida al campo, aunque la mayoría lo hicieron a los cuatro meses. La totalidad de los animales estudiados eliminaron huevos del parásito con las heces, bien en todos los muestreos o en alguno de ellos, y el número de huevos por gramo (hpg) osciló entre 67-2533 ($\bar{x}$ 244.50 $\pm$ 8.10 EE). Los valores más elevados del % de animales que eliminaban huevos (86.66%) y de la media de hpg (374.61 $\pm$ 38.5) se detectaron en febrero. En el grupo de los animales más viejos (de 7 y 8 años) se detectaron los valores más elevados del porcentaje de muestras positivas (82.86%) y de la media de hpg (372.60), y en los corderos los más bajos (57.38%, 164.37).

Palabras clave: *Dicrocoelium dendriticum*, ovinos marcados, infección natural, cinética de a eliminación de huevos, León, España.

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1. Introduction

Dicrocoeliosis, caused by the hepatic trematode *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899, is a parasitic disease of health and economic significance to extensive livestock breeding and farming. This trematode affects a wide range of species of mammals, mainly ruminants, in

Spain and various countries in Europe, Asia, America and North Africa. It can also occasionally affect humans. This parasite has an extraordinarily complex life cycle, with the intervention of various species of land molluscs and ants, which act as its primary and secondary intermediate hosts, respectively (Manga-González *et al.*, 2001). The health and financial significance of dicrocoeliosis is partly due to the direct losses caused by condemnation of infected livers and partly to the indirect costs deriving from hepatobiliary alterations caused by the parasite, such as a decrease in animal weight, delay in growth and reduced milk yield, amongst others (Cavani *et al.*, 1982; Wolff *et al.*, 1984; Jithendran & Bhat, 1996; Manga-González *et al.*, 2004). In addition, the extra costs of applying anthelmintic treatments must be considered.

Applying effective measures for the strategic control of dicrocoeliosis requires, in addition to a good diagnosis, prior study of the epidemiology, that is, the mutual relations between the parasite, the intermediate hosts, the definitive hosts and the environment. There are data available on the percentage of ovines shedding *D. dendriticum* eggs in both Spain (Del Río, 1967; Jorquera, 1967; Tarazona *et al.*, 1985; Uriarte *et al.*, 1985; García & Juste, 1987; Reina *et al.*, 1987; Ferre *et al.*, 1991, 1994; Manga *et al.* 1991; Rojo-Vázquez *et al.*, 1995) and other countries (Kalkan, 1971; Lukin, 1974; Calamel, 1976; Ambrosi & Principato, 1981; Lietava, 1984; Sotiraki *et al.*, 1999; Cringoli *et al.*, 2002; Rehbein *et al.*, 2002; Otranto & Traversa 2002). However, integrated studies on transmission of the parasite in a specific zone were scarce until our research began, possibly due to the great complexity of its biological cycle.

Bearing in mind the above, we carried out a broad integrated study in the mountains of León (NW, Spain) on transmission of the *D. dendriticum* parasite, in which we identified the mollusc and ant species -which act as first and second intermediate hosts of the parasite, respectively-, studied different aspects of their biology, distribution, behaviour and kinetic of their infection by the larval stages of *D.*

dendriticum (Manga-González *et al.*, 2001; Manga-González & González-Lanza, 2005a, 2005b). In addition, we simultaneously followed the kinetic of *D. dendriticum* egg elimination by marked lambs and ewes –definitive host-, which were grazing in the same study zone. Moreover, the influence of the environmental factors on the whole process of parasitic transmission was also taken into account.

Nevertheless, this paper only concerns the research carried out on ovines in which we intended to reveal when elimination of *D. dendriticum* eggs happens for the first time in marked lambs which went out to graze for the first time. Likewise we try to follow the egg elimination kinetic to determine the period when pasture contamination by the viable parasite eggs was high, which would favour infection of the largest number of molluscs, first intermediate host and, as a consequence, the continuation of the life cycle and parasite transmission.

2. Materials and methods

In order to monitor the elimination of *D. dendriticum* eggs by ovines grazing in the study area –the valley of Redipollos (altitude 1100 to 1400m), situated in the upper Porma basin in the province of León (NW Spain)–, faeces samples were collected from the rectum of 81 marked sheep. They were taken every month in the early morning (at 7 in summer and at 8 in winter) from June 1987 to June 1989. These animals belonged to a common flock of approximately 200: 120 ovine and the rest caprine. The animals which belonged to different farmers were taken out to graze in the morning by a shepherd and returned to their respective stables at dusk (except for some days in winter when they remained inside as the pastures were completely covered by snow). According to the verbal information given by the farmers, none of the ovines had been administered anthelmintic treatment for two years.

At the start of sampling the age of the selected animals as representatives of the whole flock was from four months to eight years. Seven groups (G) of animals were established according

to their age at the beginning of the study. A total of 35 lambs aged four to eight months were checked: G1, consisting of 22 which went out to graze for the first time in June 1987; G2, with the other 23 doing so in May of the following year. The sheep aged one year or over were divided as follows: G3, 11 one-year olds; G4, 7 aged two; G5, 11 aged three; G6, 11 aged five; and G7, 6 aged seven and eight.

The corresponding coprological analyses were carried out in the laboratory using the sedimentation method. This technique was carried out individually on 3 grams of each sample of faeces. In order to establish the number of eggs per gram (epg), the eggs in the sediment were counted using McMaster chambers. Two annual sampling periods, the first between June 1987 and May 1988 and the second between June 1988 and May 1989, were considered when drawing up the results.

To discover differences in the percentage of positive samples, among sampling months, the chi square test (χ^2) was used. A multifactorial analysis of variance was carried out to see whether there were statistically significant differences in the number of epg eliminated on the basis of three factors: age of the animals, annual sampling period and month. In order to carry out this analysis in double interactions the same age groups of the previously mentioned animals, but putting the two groups of lambs into one, were considered. In the triple interaction only three age categories were considered: 1/ animals less than one year old; 2/ from one to two years old; 3/ from three to eight years old. This was due to the non-elimination of eggs by both groups of lambs in the first sampling month, and to the lack of data from the 2-year old animals, as no samples were taken from them until October 1987 for reasons beyond our control. Pairs of epg means were compared using the test of lowest significant differences (LSD).

3. Results and discussion

3.1. Total data on *Dicrocoelium dendriticum* egg elimination by ovines

When the data obtained during the whole sampling period were taken into consideration,

100% of the animals studied shed *D. dendriticum* eggs and the number of eggs per gram (epg) oscillated between 2533 and 67, the mean ($\bar{x}$) being 244.50 ± 8.10 SE (Standard error). On considering the samples examined monthly over the whole period, the percentage of samples with *D. dendriticum* eggs was 71.44%. This value was lower than that obtained in ovines as follows: in Spain, by Del Río (1967) in the province of León (84.9%) and by García & Juste (1987) in the Basque country (100%, although, this result is referring to flocks); in France, by Calamel (1976) in the French Maritime Alp region (83.1%); in Bulgaria, by Pavlov *et al.* (1965) (90–100%); in Italy, by Ambrosi & Principato (1981) (100%); in Russia, by Vershinin (1957) (100%); in Azerbaijan, by Sadykhov & Melikov (1980) (100%). But our result was similar to those obtained: in Macedonia, by Angelovski *et al.* (1970) (75%); in Greece, by Liakos (1985) in Thessalonica (67.5%); in Turkey, by Kalkan (1971) in Kumbale (74%); in China, by Tang *et al.* (1981) in Xin Jiang (73%).

However, the positive sample percentage we recorded was higher than those obtained: in Spain, by Uriarte *et al.* (1985) in Saragossa (63.7% in an irrigated area) and by Manga *et al.* (1991) in the León mountains (63.6%); in Italy, by Quesada *et al.* (1991) in the Basilicata region (60%); in Slovenia, by Lietava (1984) (54.8%); and in Turkey, by Kalkan (1971) in Karacabey (64%). In addition, our value was much higher than those recorded: in Spain, by Reina *et al.* (1987) (4.8%) in Cáceres province, by Uriarte *et al.* (1979), 50.6% and Uriarte *et al.* (1985) (44.9% in an unirrigated zone) in the province of Saragossa, by Ferre *et al.* (1991) (7.0%) in the province of Segovia, and by Ferre *et al.* (1994) in 9 livestock farming regions of León province (26.6%); in France, by Calamel (1976) in the south-east (41.9%); in Italy, by Poglayen *et al.* (1981) in Irpinia (14.71%); in Germany, by Stuhrberg *et al.* (1975) in the Frankfurt and Oder regions (31.4%); in Austria, by Quechon (1985) in Vienna (30%); in Russia, by Bausov *et al.* (1981) in Kursk (5.9%); and in India, by Krishna *et al.* (1989) in the Kangra valley (6%).

As regards the mean number of eggs per gram, the value we found (244.50) was lower than that obtained in Spain by Manga *et al.* (1991) (323.4 epg) and in Germany by Kopp (1975) (275 epg), whilst it was higher than that recorded in Spain by Rojo *et al.* (1981) (167 epg) and Ferre *et al.* (1994) (20 epg). On considering the maximum number of epg, our value (2533) exceeded that obtained by Rojo *et al.* (1981) (773 epg) and Kalkan (1971) (685 epg), and was lower than that observed by Manga *et al.* (1991) (5340 epg).

3.2. Monthly *Dicrocoelium dendriticum* egg elimination kinetic

On considering monthly elimination of *D. dendriticum* eggs by all the animals throughout the sampling period (Fig.1A), positive elimination was detected in every month, although the highest percentages of infected animals was observed between October and February, with the maximum in the latter (86.66%) and the lowest in July (40.16%). Likewise, the highest mean epg (Fig. 1B) was obtained in February (374.65 $\pm$ 38.5) and the lowest in July (130.71 $\pm$ 12.72).

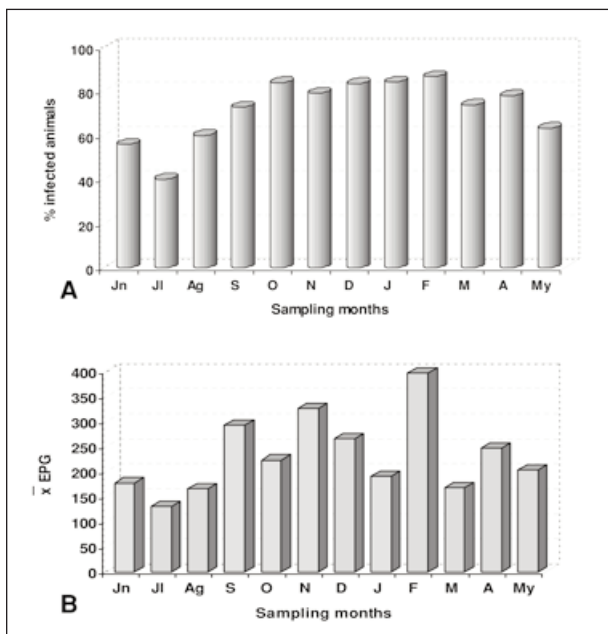


Fig. 1. (A, B). A/ Monthly percentage (%) of the total number of animals shedding *Dicrocoelium dendriticum* eggs in the two years of sampling (June 1987–May 1989). B/ Mean (0) of *D. dendriticum* eggs per gram (EPG) monthly eliminated by all the animals in the two years of sampling.

When the two annual sampling periods (June 1987–May 1988; June 1988–May 1989) were considered separately, the highest percentages of samples with *D. dendriticum* eggs were detected in February 1989 (93.65%) and in December 1987 (90.32%); and the lowest (40%) in July 1988. Likewise, the highest mean epg numbers were recorded in February 1989 (483.71 $\pm$ 55.84) and in November 1988 (434.71); and the lowest (114.43 $\pm$ 14.87) in July 1989. The maximum epg values were detected in September 1987 (2533 epg) and in February 1989 (2467 epg).

In view of our data, the mean number of eggs per gram increased in autumn and reached its maximum value at the end of winter, that is during the periods when temperatures are low (Fig. 2), which favours *D. dendriticum* egg viability in the field, as, according to Alzieu & Ducos de Lahitte (1991), they are more resistant to low temperatures than high ones. Experiments have shown that they can stand temperatures as low as -20°C to -50°C (Boray, 1985). Likewise, studies carried out under controlled field conditions in León have shown that egg mortality in faeces exposed in the environment during the hot months of July and August is nearly 100% (Alunda & Rojo-Vázquez, 1983). Our results coincide, in general terms, with those given by Manga *et al.* (1991), who observed the highest elimination at the end of autumn and in winter, and partly with those of: García & Juste (1985), who detected the highest values in spring and autumn; Euzéby (1971) who recorded them in winter and at the beginning of spring; Ambrosi & Principato (1981), who reported a large increase in autumn and maximum elimination at the end of winter and beginning of spring. However, our results do not coincide with those obtained by: Stührberg *et al.* (1975), who stated that the number of eggs excreted was higher from April to autumn and decreased in December; Kopp (1975) who reported the highest egg elimination in spring; Denev & Kostov (1984), who detected the highest values in summer and autumn-winter; and Al-Khalidi & Al-Bayati (1989), who observed them in November and May.

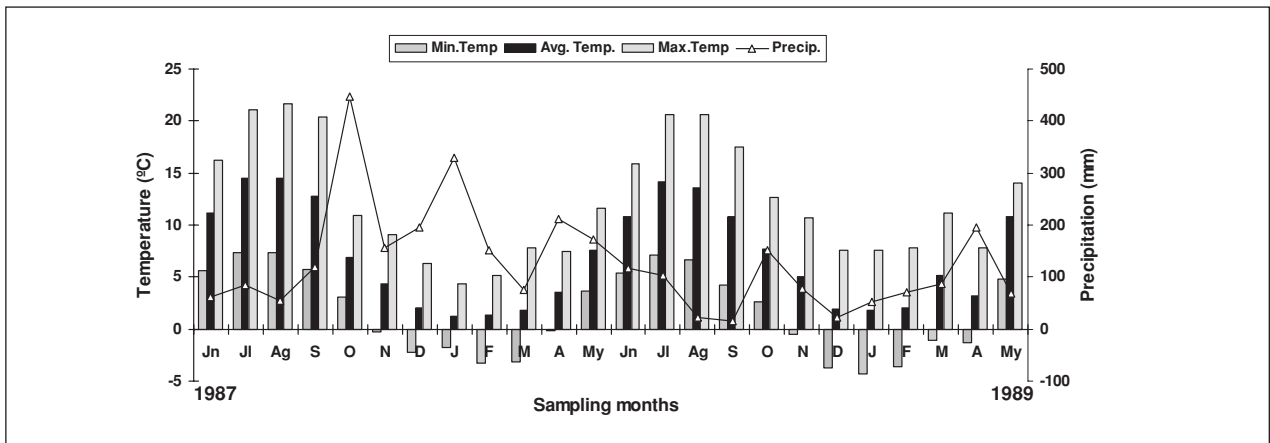


Fig. 2. Maximum, minimum and average monthly temperature and precipitation during the whole sampling period (from June 1987 to May 1989).

3.3. Elimination of eggs according to the age of the animals and the months and annual periods of sampling

When the age of the animals in the seven groups established at the start of the experiment was taken into account, the highest percentage of samples with *D. dendriticum* eggs (82.86%) was observed in group G7, consisting of animals aged 7 and 8 years, and the lowest (57.38%) in the groups of lambs (G1 and G2) aged between four and eight months. Likewise, the highest mean epg value (372.60 ± 34.25) also corresponded to the 7 and 8 year-old animals and the lowest (164.37 ± 16.63) to the lambs.

We have shown the results found in each of the seven groups of animals, established according to their age at the start of the study, separately.

In group G1 – lambs going out to graze for the first time in June 1987 – the mean number of epg for each lamb varied between 89 ± 13.91 and 256.46 ± 56.33 , whilst the absolute values oscillated between 67 and 933 epg. The highest percentage of animals shedding eggs (94.74%) was detected in October 1988. Egg elimination (Fig. 3A) began in August 1987. The highest figure for the mean epg number in the first annual sampling period was detected in November (292.38 ± 61.31), whilst it was observed in February in the second (452.5 ± 60.22).

In group G2 -lambs going out to graze for the first time in May 1988-, the mean epg of each animal oscillated between 106.80 ± 26.53 and 438.29 ± 296.15 , the absolute minimum being 67

and the maximum 2200. Egg elimination began in July 1988, when only 13.38% of the lambs were shedding eggs. The highest values (Fig. 3B) of mean epg (472.0 ± 18.64) and percentage of positive animals (100%) were detected in February 1989. On considering all the animals in the group as a whole, mean epg was (186.98 ± 9.86).

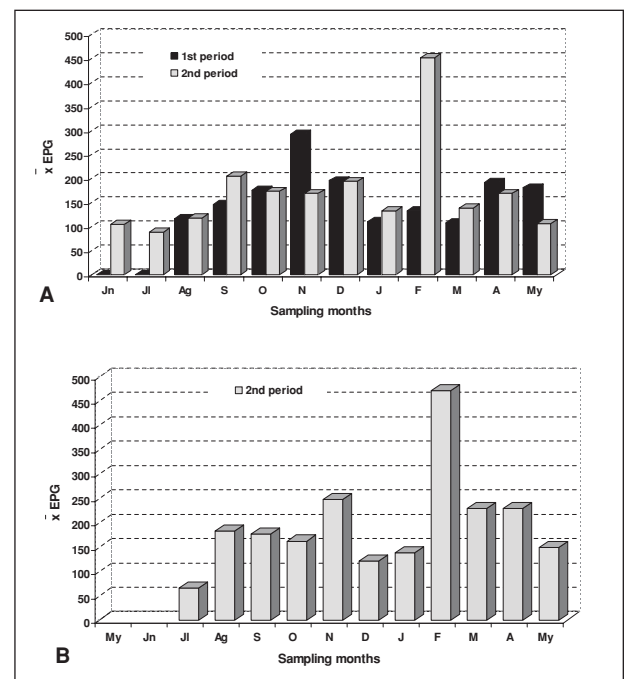


Fig. 3. (A, B). A/ Monthly mean (0) of *Dicrocoelium dendriticum* eggs per gram (EPG) shed by the G1 of lambs, which went out to graze for the first time in June 1987, considering the two sampling periods separately. First period: June 1987–May 1988. Second period: June 1988–May 1989. B/ Monthly mean (0) of *D. dendriticum* EPG shed by the G2 of lambs, which went out to graze for the first time in May 1988, referring to the second period (June 1988–May 1989).

In group G3 –animals aged one year old–, mean epg of each animal oscillated between 67.0 ± 0.0 and 520.94 ± 140.53 , whilst the absolute values varied between 67 and 2467. The highest percentage of positive samples (100%) was detected in October and December 1987, in June 1988 and in February, March and May 1989. The egg elimination kinetic we obtained in each of the two annual sampling periods (Fig. 4) was different, as occurred in the case of the lambs. In the first period, the highest mean epg value (383.37 ± 113.88) was obtained in December 1987, and in the second period in February 1989 (638.14 ± 09.84).

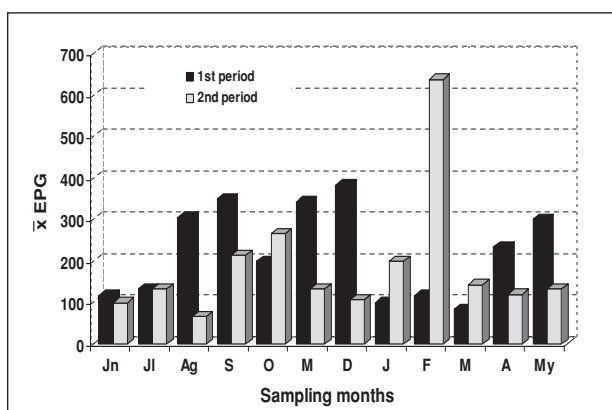


Fig. 4. Monthly mean (0) of *Dicrocoelium dendriticum* eggs per gram (EPG) shed by the G3 animals, aged one year at the start of sampling, considering the two sampling periods separately. First period: June 1987–May 1988. Second period: June 1988–May 1989.

In group G4 –two year-old animals–, no samples could be collected until October 1987. Mean epg of each animal oscillated between 105.75 ± 17.84 and 292.46 ± 65.49 , the absolute minimum epg being 67 and the maximum 800. Concerning the percentage of infection, 100% positive samples were detected in: October–December 1987; January, April, May and September 1988; and January 1989. The lowest (50%) was observed in: June–July 1988, and in April 1989. In general terms, egg elimination was highest in the first annual sampling period (Fig. 5). The highest mean epg value in that period was obtained in November 1987 (400.14 ± 82.19), whilst the highest one in the second period was observed in February 1989 (400 ± 124.51).

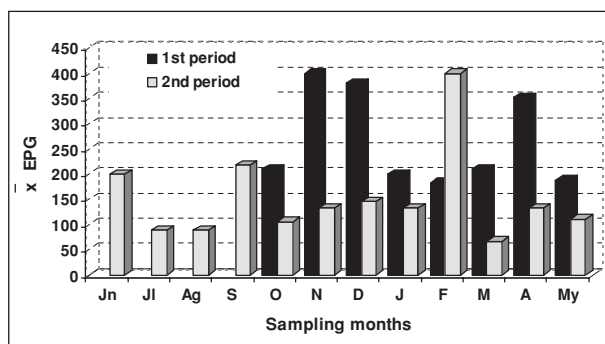


Fig. 5. Monthly mean (0) of *Dicrocoelium dendriticum* eggs per gram (EPG) shed by the G4 animals, aged two years at the start of sampling, considering the two sampling periods separately. First period: June 1987–May 1988. Second period: June 1988–May 1989.

In group G5 –three year-old animals–, the mean number of epg of each animal oscillated between 123.31 ± 9.02 and 666.71 ± 185.45 , whilst the absolute values varied from 67 to 1067. The percentage of positive samples was 100% in November–December 1987, May 1988 and February 1989. The lowest value (40%) was observed in June 1987. Egg elimination in the first annual sampling period (Fig. 6) was higher in the autumn and the highest mean epg value (673.4 ± 161.70) was observed in November 1987. As regards the second period, the highest mean epg value was observed in February (466.6 $\pm$ 110.78). In general terms, the egg elimination model in the second period was similar to that detected for the same period in the previous groups.

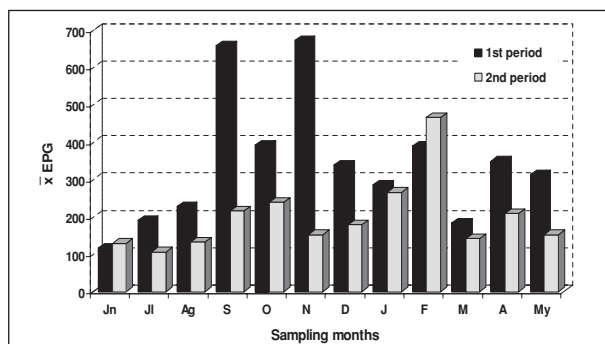


Fig. 6. Monthly mean (0) of *Dicrocoelium dendriticum* eggs per gram (EPG) shed by the G5 animals, aged three years at the start of sampling, considering the two sampling periods separately. First period: June 1987–May 1988. Second period: June 1988–May 1989.

In group G6 –five year-old animals– mean epg of each animal oscillated between $100.17 \pm$

16.16 and 460.96 ± 78.50 , the absolute minimum being 67 and the maximum 1067. Concerning the percentage of infection, 100% positive samples were detected in: September and October 1987; February, March and December 1988. The lowest percentage (44.44%) was observed in August 1988. The egg elimination kinetic (Fig. 7) in both annual sampling periods has a certain similarity to that observed in the animals in the previous group. Nevertheless, the highest mean epg value was observed in November 1987 (426.8 ± 162.87) in the first sampling year and in February 1989 (466.83 ± 97.45) in the second.

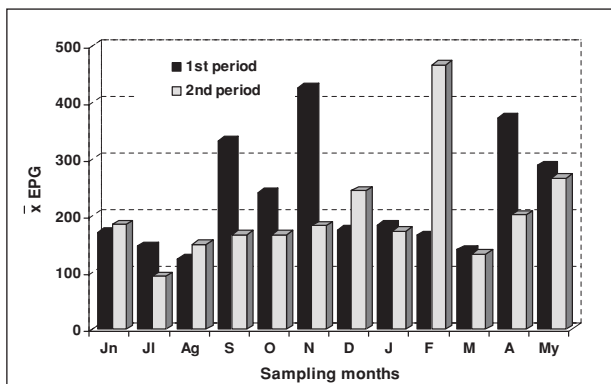


Fig. 7. Monthly mean (0) of *Dicrocoelium dendriticum* eggs per gram (EPG) shed by the G6 animals, aged four years at the start of sampling, considering the two sampling periods separately. First period: June 1987–May 1988. Second period: June 1988–May 1989.

In group G7 –seven and eight year-old animals–, mean epg of each animal oscillated between 133.50 ± 44.38 and 619.14 ± 89.02 , whilst the absolute values varied between 67 and 2133 epg. Referring to the percentage of infection, 100% positive samples were detected in: September, November and December 1987; January, April, June and October 1988; and January, February and April 1989. The lowest percentage (50%) was observed in August 1987. In the first annual sampling period (Fig. 8) mean epg was higher in autumn and at the beginning of winter, with the highest value in December (800 ± 398.68), whilst mean epg in the second period was slightly higher in winter and the highest value was detected in February (533.5 ± 118.62).

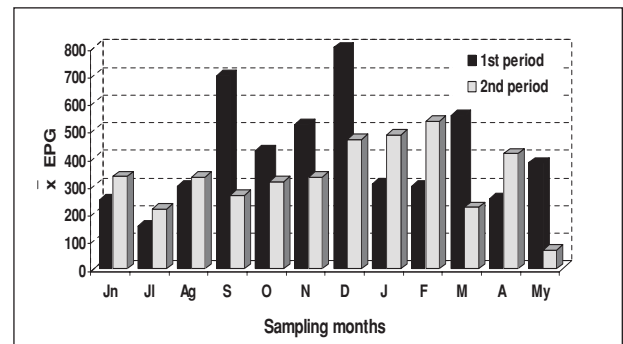


Fig. 8. Monthly mean (0) of *Dicrocoelium dendriticum* eggs per gram (EPG) shed by the G7 animals, aged seven and eight years at the start of sampling, considering the two sampling periods separately. First period: June 1987–May 1988. Second period: June 1988–May 1989.

Using chi-square analysis, statistically significant differences were observed for $P < 0.05$, in the percentage of positive samples, between the sampling months ($\chi^2 = 22.79$; $P < 0.05$; $df = 11$). On applying the 2×2 contingency tables statistically significant differences were detected for $P < 0.05$ between the value obtained in July and that for the other months, except June.

Multifactorial analysis of variance was used to study the variation in egg elimination in terms of: 1/ Age of the animals and sampling months; 2/ First and second annual period (June 1987–May 1988, June 1988–May 1989, respectively), and sampling month; and 3/ age of the animals, annual period and season. In this way statistically significant differences were detected as regards epg value between: the different age groups ($F = 11.34$; $P < 0.001$; $df = 5$), sampling months ($F = 6.81$; $P < 0.001$; $df = 11$), annual periods ($F = 13.32$; $P < 0.001$; $df = 1$) and the age-annual period ($F = 3.89$; $P < 0.001$; $df = 5$ and 984) and season-annual period ($F = 7.58$; $P < 0.001$; $df = 3$) double interactions. No statistically significant differences were detected in the triple age-period-season interaction or in the age-season interaction.

When compared by pairs, using the LSD test, the mean epg values obtained on analysing the samples collected monthly, statistically significant differences were observed for $P < 0.05$ between: 1/ February and the rest of the months, except November; 2/ November and all the other months, except September and December; 3/ September and

the rest of the months, except April, October, November and December. In the same way, the LSD test was used to compare the mean epg values, obtained on analysing the samples grouped according to the animals' ages, by pairs. Statistically significant differences were thus observed for $P < 0.05$, between groups G7 (7–8 year olds) and G5 (3 year olds) and the rest of the groups, respectively.

Our results, as regards age, seem to coincide with those obtained by other authors (in unmarked animals), who confirm that age clearly influenced egg elimination, in such a way that the highest infection rates are in the oldest animals (Vershinin, 1957; Dementev, 1968; Kalkan, 1971; Kopp, 1975; Al-Khalidi & Al-Bayati, 1989). However, Manga *et al.* (1991) found no influence by animal age on the infection prevalence and the mean number of eggs per gram eliminated. The low infection rate we found in the group of lambs coincides with the results of Dementev (1968), who reported that dicrocoeliosis in Kazakhstan did not affect animals under four months old. Lukin (1974) observed 100% infection in one year-old animals, in Russia.

If we bear in mind the data obtained in both groups of lambs, elimination of *D. dendriticum* eggs began two months after they first went out to graze. This coincides with the prepatency period given by various authors (Svadzhyan, 1956; Grigoryan & Akopyan, 1960; Salimov, 1972; Chandra, 1973; Campo *et al.* 2000, amongst others). Nevertheless, elimination started four months after the lambs went out to graze (16 months in the extreme case) in most of the animals. From that moment until the sampling period ended the elimination model was similar for both groups of lambs in the second year, detecting the maximum mean epg value in February, whilst in the previous year the highest figure for mean epg in the first group was detected in November. Our results on the time when egg elimination began coincide, in general terms, with what was reported by Vershinin (1957), who stated that the lambs born in winter in Russia were infected with *D. dendriticum* in May, and started to eliminate eggs at the beginning of

July, and by Kalkan (1971) who recorded that the lambs born in winter in Turkey were infected in May and June and started to eliminate *D. dendriticum* eggs in August and November in Karacabey and Kumkale, respectively. In addition, for this author, the number of eggs per gram gradually increased and was highest at the end of the year. Nicolas *et al.* (1985) recorded the highest value in lambs in Haute-Vienne, in November.

According to our results, some lambs from both groups started the *D. dendriticum* egg elimination two months after they first went out to graze in June and May, respectively. This is normal if we take into account the prevalence period and the fact that we have observed, in the same area, active ants between March and November and infected ones with *D. dendriticum* metacercariae from April to November, in those collected from the nest, and in tetania between May and October (Manga-González *et al.*, 2001; 2005a). In the following months and until November, when ant hibernation starts, the ingestion of ants containing infective metacercariae by the ovines and the number of adults worms of *D. dendriticum* in their liver increase. As consequence, parasite egg elimination reaches the highest values at the end of autumn and mainly in winter, period when the temperature are low, which favours *D. dendriticum* egg viability in the field. Due to this, it would be convenient to give twice anthelmintic treatments to sheep, one in autumn and other in winter, in order to interrupt egg elimination and, therefore, to avoid pasture contamination by viable parasite eggs. This will reduce the probability of mollusc intermediate hosts to be infected in spring, just when they start to be actives and are very abundant (Manga-González *et al.*, 2001).

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