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Original article

Fasciola hepatica and *Paramphistomum daubneyi*: changes in prevalences of natural infections in cattle and in *Lymnaea truncatula* from central France over the past 12 years

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Abstract – A retrospective study was carried out over a 10- to 12-year period to analyse the changes in prevalences of natural fasciolosis and paramphistomosis among cattle and snails in central France, and to determine the causes which had induced these changes. The prevalences of natural fasciolosis in cattle increased from 1990 to 1993 (13.6% to 25.2%) and diminished afterwards up to 1999 (at 12.6%). Those of natural paramphistomosis showed a progressive increase between 1990 and 1999 (from 5.2 to 44.7%). The prevalences of natural infections and the numbers of free rediae counted in the snails (*Lymnaea truncatula*) infected with *F. hepatica* did not show any significant variations over time. By contrast, the prevalences of natural paramphistomosis in snails significantly increased from 1989 to 1996 and remained afterwards in the same range of values (3.7–5.3%), while the number of free rediae significantly increased up to 2000 (from a mean of 6.5 to 13.8 rediae per infected snail, respectively). Three hypotheses may explain the increase of paramphistomosis in cattle and snails: a better quality of diagnosis for the detection of *P. daubneyi* eggs in veterinary analysis laboratories, the use of specific molecules in the treatment of cattle fasciolosis since 1993, and the lack of an effective treatment up to now against cattle paramphistomosis. Since the objective of most farmers in central France is to obtain the highest antiparasitic efficiency with a single treatment of cattle per year, it is reasonable to assume that the prevalence of bovine paramphistomosis will continue to increase in the future.

cattle / fasciolosis / Limousin region / *Lymnaea truncatula* / paramphistomosis

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Résumé – *Fasciola hepatica* et *Paramphistomum daubneyi* : changements dans les prévalences des infestations naturelles chez les bovins et chez *Lymnaea truncatula* dans le centre de la France au cours des 12 dernières années. Une étude rétrospective a été réalisée sur une période de 10 à 12 années pour analyser les modifications qui se sont produites dans les prévalences de la fasciolose et de la paramphistomose naturelles chez les bovins et les limnées du centre de la France, et pour déterminer les causes qui ont provoqué ces changements. Chez les bovins, la prévalence de la fasciolose naturelle s'est accrue entre 1990 et 1993 (de 13,6 à 25,2 %) avant de diminuer par la suite jusqu'en 1999 (à 12,6 %). Celle de la paramphistomose naturelle a augmenté de manière progressive entre 1990 et 1999 (de 5,2 à 44,7 %). La prévalence de l'infestation naturelle avec *F. hepatica* et le nombre de rédies libres chez *Lymnaea truncatula* n'ont pas montré de variations significatives dans le temps. Par contre, la prévalence de l'infestation naturelle avec *Paramphistomum daubneyi* chez les limnées a présenté une augmentation significative entre 1989 et 1996 avant de rester ensuite dans la même gamme de valeurs (3,7–5,3 %), tandis que le nombre de rédies libres a augmenté jusqu'en 2000 (de 6,5 à 13,8 rédies en moyenne par mollusque infesté). Trois hypothèses peuvent expliquer cet accroissement de la paramphistomose chez les bovins et les mollusques : une meilleure qualité dans le diagnostic des œufs de *P. daubneyi* au niveau des laboratoires d'analyses vétérinaires, l'emploi de molécules spécifiques dans le traitement de la fasciolose chez les bovins depuis 1993, et le manque actuel d'un traitement efficace contre la paramphistomose. Comme l'objectif de la plupart des éleveurs dans le centre de la France est d'obtenir la meilleure efficacité antiparasitaire avec un seul traitement annuel, il est raisonnable de penser que la prévalence de la paramphistomose bovine continuera d'augmenter dans l'avenir.

bétail / fasciolose / région du Limousin / *Lymnaea truncatula* / paramphistomose

1. INTRODUCTION

Fasciolosis and paramphistomosis are two important parasitoses of farm livestock. Contrary to the former disease which results from a single helminth species (*Fasciola hepatica*) in European countries, paramphistomosis may be caused by different species of *Paramphistomum* (*P. cervi*, *P. daubneyi*, *P. ichikawai*, or *P. microbothrium* in France, for example [19]). If fasciolosis is well known for its world-wide distribution and the serious economic losses the parasite causes in the animal husbandry industry, paramphistomosis is still misjudged, as most reports on this disease do not quote the responsible species of *Paramphistomum* and the various species of the family Paramphistomidae are difficult to identify from the systematic point of view [13]. In western Europe, the most frequent paramphistomid species affecting cattle seems to be *P. daubneyi* [10, 19]. However, since the first description of the parasite only dates back to 1962 [9], the distribution of this last paramphistomid in the world is still imprecise (in some countries of Europe and eastern Africa) and the list of the definitive hosts is limited to several species of ruminants [18, 19].

Great variations in prevalences of natural fasciolosis in cattle can be noted in relation to the method used to detect fluke infection and the country in which investigations were performed. In a review by Torgerson and Claxton [23], these prevalences ranged from 0.95% up to 94% between 1989 and 1995. In more recent studies, similar variations could be noted, with prevalences ranging from 1.3% in Venezuela [8] (out of 763 065 cattle slaughtered) up to 51.2% in Ethiopia [24] (out of 4 730 cattle) or 100% in Bolivia [15] (out of 5 191 cattle). In France, the most important set of results concerns the Limousin region, with a global prevalence of 13.0% [14] out of 51 555 cattle examined. On the contrary to fasciolosis, the data on the prevalences of

P. daubneyi infections in cattle are scarce, and are recorded from investigations performed on slaughtered cattle: 5.4% (out of 1 244) [7], or 24.6% (out of 1 741) [22] in France, for example.

The same remarks may be made for the prevalences of natural infections in *Lymnaea truncatula*, which is the intermediate host of *F. hepatica* and *P. daubneyi* in most European countries [1]. With *F. hepatica*, the most complete set of results has been reported by Ollerenshaw [16] in Great-Britain and involved the collection and dissection of over 52 000 *L. truncatula* between 1960 and 1969. According to this author, prevalence is usually less than 2% and can increase up to 20% or occasionally to a higher value when conditions are favourable for the hatching of the fluke eggs. On the contrary to fasciolosis, there was no report on the infection of snails with *P. daubneyi* in France before 1990. The only reports published after this date originated from several studies performed by Szmidt-Adjidé et al. [21] (a prevalence of 4.4% out of 2 798 dissected *L. truncatula*), or by Abrous [1] or Abrous et al. [4] (9.8% out of 2 703 snails).

According to Dorchies [10, 11], the detection of paramphistomosis among French cattle has increased over the past 20 years. In view of this increasing detection in France, the three following questions arose: what were the changes in the prevalences and intensities of natural *F. hepatica* and *P. daubneyi* infections in cattle during the past 12 years? Did these changes have any consequences on the infection of the intermediate host over time? What were the causes of these changes? To answer these questions, a retrospective study was carried out to analyse the results obtained from 1989 to 2000 on the natural infection of cattle and snails by these parasites in the Limousin region (central France), and to determine the causes which induced these changes.

2. MATERIALS AND METHODS

2.1. Investigations in cattle

A total of 12 389 faecal samples, originating from different cattle-breeding farms located in the department of Corrèze (central France), were examined between 1990 and 1999 using a coproscopic technique [6]. The first parameter studied was the annual prevalences of natural infections with *F. hepatica* or *P. daubneyi* in cattle. To determine the existence of seasonal variations in these natural infections, two other parameters were also considered: the monthly prevalences of *F. hepatica* or *P. daubneyi* infections over these 10 years, and the mean numbers of trematode eggs per gram of faeces. Mean values and standard deviations were determined for each of the last two parameters. A Spearman correlation test (r_s) and a one-way analysis of variance [20] were used to determine the levels of significance.

2.2. Investigations in snails

The study was carried out between 1989 and 2000 on a total of 141 farms located in the departments of Corrèze, Creuse, and Haute Vienne (central France). These farms were chosen either randomly among a sample of 407 cattle-breeding farms [3] surveyed in these three departments, or following the detection of fasciolosis (since 1989) or paramphistomosis (since 1994) among cattle. Every year, the sampling of *L. truncatula* was carried out among a sample of 8 to 12 farms (out of 141). Each farm was only studied for one year, because the results found in snails led the breeders to request the detection of trematode infections in their ruminants and also the treatment. On these 141 farms, the sampling of *L. truncatula* was performed in June-July and in September-October. The choice of these two periods was based on the fact that most cercarial sheddings from naturally-infected snails occur during these months in central France and are thus risk periods for ruminants [17]. The collected snails were over 4 mm in height. They were collected in the immersed zones of each area as well as in stagnant or shallow waters (no more than five cm in depth during the study period). The search for snails in each habitat and subsequent collection were made by two people for 30 to 40 min.

The snails were dissected under a stereomicroscope to detect trematode larval forms and to classify infected snails into four groups: snails infected with *F. hepatica* only, *P. daubneyi* only, both, or snails infected by other trematode species. The most differentiated larval forms of *F. hepatica* and/or *P. daubneyi* found in most infected snails were cercaria-containing rediae and free cercariae; in the other snails, immature rediae were noted. Cercariae-containing rediae of *F. hepatica* possess a well-developed pharynx, collar rings and pairs of appendages in the third posterior part of their bodies [5]. In contrast, the rediae of *P. daubneyi* are shorter with a small pharynx, and their bodies lacked a collar and appendages [2]. Free-cercariae of *F. hepatica* are white-coloured and quick swimming, whereas those of *P. daubneyi* are dark brown and sluggish. A subsequent count of free rediae was finally performed if the snails were infected with *F. hepatica* and/or *P. daubneyi*.

The global prevalences of natural infections with *F. hepatica* in *L. truncatula* were calculated by adding the number of snails harbouring only larval forms of *F. hepatica* and that of concurrently infected snails, and dividing the figure obtained by the total number of snails examined. A similar method was used to determine global prevalence of natural infections with *P. daubneyi*. The last parameter was the number of free rediae counted in snails infected with *F. hepatica* and/or *P. daubneyi*. A Spearman correlation test (r_s), a comparison test of experimental frequencies, and a one-way analysis of variance [20] were used to determine the levels of significance.

3. RESULTS

3.1. Natural infections in cattle

The mean prevalences of *F. hepatica* and *P. daubneyi* infections, recorded between 1990 and 1999 (Tab. I), were 17.1% and

Table I. Annual variations in the prevalences of natural infections with *Paramphistomum daubneyi* and *Fasciola hepatica* in cattle in the department of Corrèze (central France).

Year	Number of coproscopic examinations	Prevalences of natural infections in %	
		Fasciola hepatica	*Paramphistomum daubneyi*
1990	879	13.61	5.23
1991	1262	20.00	6.73
1992	1140	23.41	8.71
1993	1296	25.25	12.58
1994	1146	21.51	20.86
1995	1381	17.74	24.13
1996	1234	11.96	23.54
1997	1406	11.28	33.24
1998	1315	14.18	43.63
1999	1310	12.60	44.72
Total	12 389	17.1 ± 4.8*	22.3 ± 13.7*

* Mean prevalences ± S.D.

22.3%, respectively. However, great variations of natural infections in relation with years could be noted. The prevalence of infections with *F. hepatica* increased from 1990 to 1993 (from 13.6% to 25.2%), diminished up to 1996 (to 11.9%), and did not much vary until 1999. However, the variation of these prevalences over the past 10 years was not significant. On the contrary of fasciolosis, the prevalence of natural infections with *P. daubneyi* significantly increased ($r_s = 0.84$, $P < 0.01$) from 5.2% (1990) to 44.7% (1999) and a steep increase in the frequency of positive examinations since 1995 could be noted.

Figure 1 gives the seasonal variations in the monthly prevalences of these natural infections and the number of trematode eggs found in cattle faeces. The lowest prevalences of fasciolosis (Fig. 1a) were noted in July and August. Afterwards, there was an increase in the number of positive examinations from

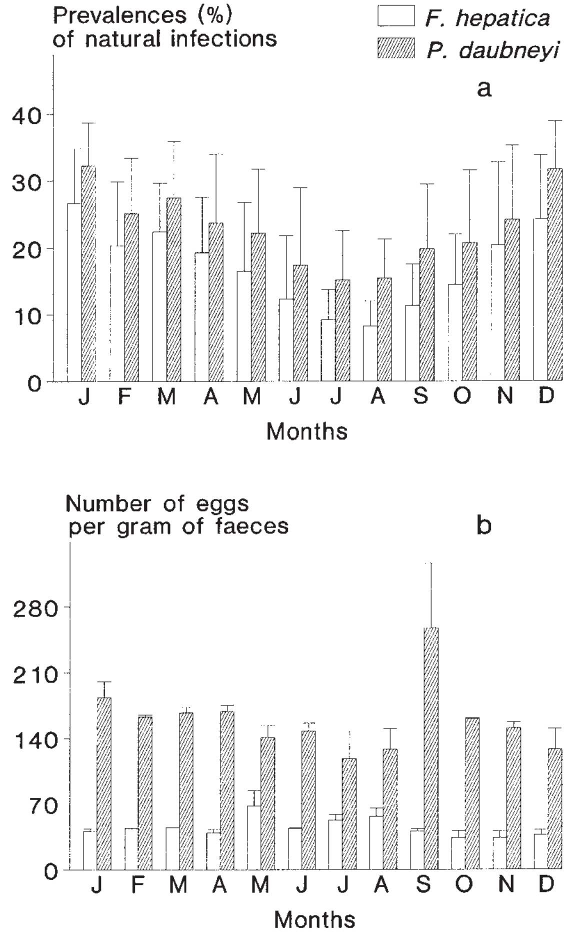

Figure 1. Mean monthly prevalences of natural infections (a) and monthly mean numbers of *Fasciola hepatica* and *Paramphistomum daubneyi* eggs excreted in the faeces of cattle (b) originating from the Corrèze department (central France) throughout the past 10 years (1990–1999).

September to January. Similar results were noted for the monthly prevalences of *P. daubneyi* infections. The mean monthly number of *F. hepatica* eggs (Fig. 1b) was less than 50 per gram of faeces, whereas that of *P. daubneyi* eggs ranged from 100 to 250 per gram of faeces. A significant difference ($F = 44.5$, $P < 0.001$) between the mean monthly numbers of *F. hepatica* eggs and those of *P. daubneyi* eggs could be noted. However, for each trematode considered separately, there was no significant variation between the monthly numbers of eggs throughout the year.

3.2. Natural infections in snails

Table II gives global prevalences of natural infections with *F. hepatica*, *P. daubneyi*, or other trematodes in *L. truncatula* dissected between 1989 and 2000. First, the prevalences of natural fasciolosis in snails ranged from 4.7% (1989) to 7.2% (1993), and slowly decreased after that date reaching 3.3% (in 2000). However, the variation of these prevalences over the past 12 years was not significant. On the contrary to fasciolosis, the prevalences of natural paramphistomosis in *L. truncatula* significantly increased ($r_s = 0.71$, $P < 0.05$) with time from 1989 to 1996. Afterwards, the values of prevalences were in the same range (from 3.7% to 5.3%) and did not show any significant variation.

The numbers of free rediae found in snails infected with *F. hepatica* or *P. daubneyi* are given in Table III. The mean redial burden of *F. hepatica* ranged from 12.3 to 17.1 per infected snail and did not show any significant variation, whatever the year of study. By contrast, in the case of *P. daubneyi*, there was a significant increase ($r_s = 0.57$, $P < 0.05$) in the number of free rediae from 1990 to 2000 (from a mean of 6.5 to 13.8 rediae per infected snail).

4. DISCUSSION

Under the conditions of cattle breeding in the Limousin region, the prevalence of natural fasciolosis did not vary very much between

Table II. Prevalences of trematode infections in *Lymnaea truncatula* collected on 141 farms of the Limousin region (central France) in relation to the year of study.

Year of study	No. of *L. truncatula* dissected	Prevalence (%) of snails naturally infected with	
		Fasciola hepatica	*Paramphistomum daubneyi*
1989	654	4.7	0
1990	798	5.1	0.8
1991	668	5.8	1.7
1992	934	6.8	2.1
1993	2 072	7.2	4.3
1994	1 543	5.7	3.0
1995	1 347	4.1	3.5
1996	2 477	6.6	6.3
1997	1 843	4.8	4.2
1998	1 475	4.1	3.7
1999	2 198	3.5	4.8
2000	2 782	3.3	5.3
Total	18 791	5.1 ± 1.2*	3.3 ± 1.7*

* Mean prevalences ± S.D.

Table III. Number of free rediae in the body of *Lymnaea truncatula* infected with *Fasciola hepatica* or *Paramphistomum daubneyi* in relation to the year of study.

Year of study	Snails infected with *Fasciola hepatica*		Snails infected with *Paramphistomum daubneyi*	
	Total number of snails	Number of free rediae*	Total number of snails	Number of free rediae*
1989	31	15.2 ±2.9	0	–
1990	41	14.3 ±3.2	7	6.5 ±2.1
1991	39	16.7 ±4.3	12	6.8 ±3.5
1992	64	12.3 ±2.7	20	7.2 ±3.0
1993	149	14.8 ±3.0	89	7.7 ±2.9
1994	88	17.1 ±5.1	47	8.5 ±3.5
1995	56	13.7 ±5.3	48	8.8 ±2.0
1996	164	15.6 ±5.1	156	9.5 ±3.7
1997	89	13.8 ±3.4	78	11.5 ±4.2
1998	61	15.0 ±4.5	55	13.5 ±2.3
1999	77	16.7 ±4.5	106	14.3 ±3.2
2000	92	13.8 ±3.7	148	13.8 ±2.7

* Mean values ± S.D.

1994 and 1999, and this result was the consequence of the anthelmintic treatments performed by breeders during this period. Conversely, the increase in the prevalence of natural paramphistomosis sets this parasitosis in the top of local parasitic infections and this situation is difficult to comment. Since the difficulty to identify the eggs of *F. hepatica* and *P. daubneyi* in cattle faeces during coproscopic examinations [10] had already been reported, an assumption would be to admit a better quality of diagnosis for the detection of *P. daubneyi* eggs in veterinary analysis laboratories. However, since the larval forms of *P. daubneyi* were scarcely found in the *L. truncatula* collected in central France (four infected snails out of 3 547 collected in the field between 1970 and 1980, unpublished data), the difficulty in the identification of trematode eggs is not the single factor and it is logical to think that other causes may be evoked to explain these changes in the prevalences of natural infections. In our opinion, two perhaps complementary hypotheses might be considered. The first concerns the treatment of fasciolosis in cattle-breeding farms: the use of specific molecules against *F. hepatica* since 1990 would have allowed the development of paramphistomosis since 1993 in these farms, whereas that of broad-spectrum anthelmintics before 1990 would have allowed the control of this last infection. The second hypothesis would be the lack of an effective treatment up to now against *P. daubneyi*.

On the contrary to the snails infected with *F. hepatica* for which the characteristics of natural infections did not show any significant variations over time, an increase in the prevalences of natural paramphistomosis in *L. truncatula* was noted between 1989 and 1996, while a more progressive increase in the numbers of *P. daubneyi* rediae was found between 1990 and 2000. These changes in the natural infections of snails with *P. daubneyi* agreed with the previous results found in cattle and confirm the extension of paramphistomosis in France [10, 11]. However, two points warrant special comments. The first concerns the prevalences of natural paramphistomosis among cattle and snails over the period of time studied. If the values of this parameter in cattle steadily increased up to 44.7% from 1990 to 1999 (see Tab. I), the corresponding prevalences in snails only increased from 1990 to 1997 and remained similar afterwards. To explain this difference, the most valid hypothesis is to admit that the more limited prevalences of infections in snails would result in favourable periods (in the spring particularly) and in the best sizes for snail infection (the 4-mm high snails are the most susceptible to infection [12]). The second point was the increase of the redial burden in snails infected with *P. daubneyi* over time. This last finding suggested a progressive adaptation of this parasite to this intermediate host, so that the parasite burden within the snails increased over time.

In conclusion, the treatment of fasciolosis during the past 10 years did not effectively decrease the prevalences of *F. hepatica* infections in cattle and, subsequently, in snails. On the contrary, the absence of an effective treatment against *P. daubneyi* infections had resulted in a clear increase of prevalences in cattle and snails. Since the objective of most farmers in the department of Corrèze was to obtain the highest antiparasitic efficiency with a single treatment of cattle per year (unpublished results), it is reasonable to assume that the prevalence of bovine paramphistomosis in central France will continue to increase in the future.

REFERENCES

[1] Abrous M., Les mollusques hôtes et les formes larvaires de *Paramphistomum daubneyi* Dinnik, 1962 (Trematoda) dans le centre de la France. Influence d'une co-infestation avec *Fasciola hepatica* Linné, 1758. Thèse Doct. Univ. Limoges, Limoges, 1999.

[2] Abrous M., Rondelaud D., Dreyfuss G., *Paramphistomum daubneyi*: the development of redial generations in the snail *Lymnaea truncatula*, Parasitol. Res. 83 (1997) 64-69.

[3] Abrous M., Rondelaud D., Dreyfuss G., Cabaret J., Unusual transmission of the liver fluke, *Fasciola hepatica*, by *Lymnaea glabra* or *Planorbis leucostoma* in France, J. Parasitol. 84 (1998) 1257-1259.

[4] Abrous M., Rondelaud D., Dreyfuss G., Cabaret J., Infection of *Lymnaea truncatula* and *Lymnaea glabra* by *Fasciola hepatica* and *Paramphistomum daubneyi* in farms in central France, Vet. Res. 30 (1999) 113-118.

[5] Augot D., Rondelaud D., Dreyfuss G., Cabaret J., Bayssade-Dufour C., Albaret J.L., Characterization of *Fasciola hepatica* redial generations (Trematoda: Fasciolidae) by morphometry and chaetotaxy under experimental conditions, J. Helminthol. 72 (1998) 193-198.

[6] Calamel M., Soulé C., Technique coproscopique standard appliquée au diagnostic des maladies parasitaires, Recl. Méd. Vét. 151 (1975) 299-303.

[7] Casset I., Enquête sur la paramphistomose bovine: recherche des parasites en abattoir, Rev. Méd. Vét. (Toulouse) 140 (1989) 925-927.

[8] Chirinos A., Chirinos N. de, Roman R., Homez G., Pirela H., Rodriguez N., Distomatosis hepatica bovina a nivel de dos mataderos industriales del Estado de Zulio, Venezuela, Revista Cientifica, Facultad de Ciencias Veterinarias, Universidad de Zulia 10 (2000) 297-302.

[9] Dinnik J.A., *Paramphistomum daubneyi* nov. sp. from cattle and its snail host in Kenya Highland, Parasitology 42 (1962) 143-151.

[10] Dorchies P., Les Paramphistomidés : leur apparente extension en France et les difficultés pratiques d'identification en coproscopie, Rev. Méd. Vét. (Toulouse) 140 (1989) 573-577.

[11] Dorchies P., La paramphistomose bovine en France : une parasitose en extension, Bull. Mens. Soc. Vét. Prat. Fr. 82 (1998) 423-438.

[12] Gold D., Goldberg M., Temperature effect on susceptibility of four species of *Lymnaea* snails to infection with *Fasciola hepatica* (Trematoda), Isr. J. Zool. 28 (1979) 193-198.

[13] Horak I.G., Paramphistomiasis of domestic ruminants, Adv. Parasitol. 9 (1971) 33-72.

[14] Mage C., Épidémiologie de l'infestation par *Fasciola hepatica* chez les bovins en Limousin (France), Rev. Méd. Vét. (Toulouse) 140 (1989) 407-411.

[15] Mas-Coma S., Angles R., Esteban J.G., Bargues M.D., Buchon P., Franken M., Strauss M., The northern Bolivian Altiplano: a region highly endemic for human fasciolosis, Trop. Med. Int. Health 4 (1999) 454-467.

[16] Ollerenshaw C.B., Some observations on the epidemiology of fascioliasis in relation to the timing of molluscicide applications in the control of the disease, Vet. Rec. 88 (1971) 152-164.

[17] Rondelaud D., Dreyfuss G., Variability of *Fasciola* infection in *Lymnaea truncatula* as a

function of snail generation and snail activity, J. Helminthol. 71 (1997) 161-166.

[18] Sey O., On the species of *Paramphistomum* of cattle and sheep in Hungary, Acta Vet. Acad. Sci. Hung. 24 (1974) 19-37.

[19] Sey O., Revision of the Amphistomes of European ruminants, Parasit. Hung. 13 (1980) 13-25.

[20] Stat-Itcf, Manuel d'utilisation. Institut des céréales et des fourrages, Service des études statistiques, Boigneville, France, 1988.

[21] Szmidt-Adjidé V., Rondelaud D., Dreyfuss G., Premières données sur l'infestation naturelle de *Lymnaea truncatula* Müller par *Paramphistomum daubneyi* Dinnik dans le département de la Haute-Vienne, Bull. Soc. Fr. Parasitol. 12 (1994) 183-188.

[22] Szmidt-Adjidé V., Abrous M., Adjidé C.C., Dreyfuss G., Lecompte A., Cabaret J., Rondelaud D., Prevalence of *Paramphistomum daubneyi* infection in cattle in central France, Vet. Parasitol. 87 (2000) 133-138.

[23] Torgerson P., Claxton J., Epidemiology and control. Chapter 4, in: Dalton J.P. (Ed.), Fasciolosis, CABI Publishing, Oxon, UK, 1999, pp. 113-149.

[24] Yilma J.M., Mesfin A., Dry season bovine fasciolosis in northwestern part of Ethiopia, Rev. Méd. Vét. (Toulouse) 151 (2000) 493-500.