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Original article

Myocastor coypus as a reservoir host of *Fasciola hepatica* in France

Alexandre MÉNARD[a], Albert AGOULON[a], Monique L'HOSTIS[a], Daniel RONDELAUD[b], Stéphane COLLARD[a], Alain CHAUVIN[a]*

[a] UMR ENVN /INRA 1034 "Interactions Hôte-Parasite-Milieu", École Nationale Vétérinaire de Nantes, BP 40706, 44307 Nantes Cedex 03, France
[b] Faculté de Pharmacie, Université de Limoges, 2 rue du Docteur Raymond Marcland, 87000 Limoges, France

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Abstract – To clarify the role of the nutria *Myocastor coypus* in the epidemiology of domestic fasciolosis in Loire-Atlantique (department of western France), 438 nutrias were trapped in 9 humid areas of the department and 304 nutrias were trapped in 3 farms where *Fasciola hepatica* was present; all animals were necropsied. Liver flukes were found in 160 nutrias: 38 nutrias randomly taken in the department (8.7%) and 122 trapped in fasciolosis areas (40.1%). The average parasitic burden was 5.7 flukes per nutria. Sixty-five percent of the liver flukes measured more than 18 mm (size of sexual maturity). The coproscopic examinations carried out on 144 infected nutrias showed that 90% of the infected nutrias shed fluke eggs. The hatching rate was 39.6%. Two groups of 100 *Lymnaea truncatula* snails, originating from 2 different populations, were exposed to *F. hepatica* miracidiae hatched from eggs collected from infected nutrias. The prevalence of the infection was 74% and 58.6% in the 2 groups of snails. The average redial burden was 6.2 rediae per snail. The total number of metacercariae was 72.4 metacercariae per snail producing cercariae. Two groups of 5 sheep were orally infected by 150 metacercariae of nutria or sheep origin, respectively. The installation rates of *F. hepatica* in sheep were respectively 31.6% and 29.6% for the two groups. Specific antibody kinetics of sheep were similar whether the metacercariae were of nutria or sheep origin. *M. coypus* allows the complete development of *F. hepatica* and releases parasitic elements that are infective for domestic ruminants. Because of its eco-ethologic characteristics, the nutria could be a potential wild reservoir of *F. hepatica* in France.

epidemiology / *Fasciola hepatica* / wild fauna / *Myocastor coypus* / rodent

Résumé – Le ragondin, hôte réservoir de *Fasciola hepatica* en France. Pour préciser le rôle du ragondin *Myocastor coypus* dans l'épidémiologie de la fasciolose domestique en Loire-Atlantique (département de l'ouest de la France), 438 ragondins provenant de 9 zones humides du département

* Correspondence and reprints
Tel: (33)-240 68 76 98; fax: (33)-240 68 77 51; e-mail: chauvin@vet-nantes.fr

et 304 ragondins provenant de 3 fermes atteintes de fasciolose bovine ont été prélevés et autopsiés. Les douves isolées ont été comptées et mesurées. Des douves hépatiques ont été mises en évidence chez 160 ragondins : 38 ragondins prélevés au hasard dans le département (8,7 %) et 122 prélevés dans des zones fasciologènes (40,1 %). L'intensité parasitaire moyenne était de 5,7 douves/ragondin et 65 % des douves isolées mesuraient plus de 18 mm (taille de la maturité sexuelle). Des examens coproscopiques (n = 144) ont montré que 90 % des ragondins infestés excrétaient des œufs de *Fasciola hepatica*. Le taux d'éclosabilité des œufs extraits de la bile était de 39,6 %. Deux groupes de 100 limnées tronquées provenant de 2 populations différentes ont été exposés aux miracidiums de *F. hepatica* obtenus après éclosion des œufs prélevés chez des ragondins infestés. La prévalence de l'infestation était de 74 % et 58,6 % chez les mollusques des 2 groupes. La charge rédienne moyenne était de 6,2 rédies / limnée et le nombre total de métacercaires était de 72,4 métacercaires / limnée produisant des cercaires. Deux groupes de 5 moutons ont été infestés oralement par 150 métacercaires d'origine Ragondin ou Mouton. Les taux d'installation de *F. hepatica* étaient respectivement de 31,6 % et 29,6 % pour les deux groupes. Les cinétiques d'anticorps étaient identiques pour les deux groupes. *M. coypus* est donc capable d'assurer le développement complet du parasite et libère des éléments parasitaires infestants pour les ruminants domestiques sympatriques. Ses caractéristiques éco-éthologiques font de lui un réservoir sauvage potentiel de *F. hepatica* en France.

épidémiologie / *Fasciola hepatica* / faune sauvage / *Myocastor coypus* / rongeur

1. INTRODUCTION

Fasciolosis is an important parasitic disease of domestic ruminants throughout the world. The infection causes severe economic losses due to mortality, liver condemnation, reduction in milk and meat production [16, 17]. Wild herbivorous mammals are also susceptible to *Fasciola hepatica* infection [19, 20] but the role of these species in the epidemiology of fasciolosis is discussed. Lagomorphs and marsupials have been described as reservoirs of *F. hepatica* [3, 29]. The black rat *Rattus rattus* has been described as a reservoir of fasciolosis only in specific biotopes [31]. Brown rats (*Rattus norvegicus*) and mice (*Mus musculus*) are commonly used as definitive hosts for experimental purposes; but they seem to be only sporadically naturally infected: only one mouse infection has been described, in Corsica [19], and brown rat infection has been observed in Iraq [22]. In France, the nutria (*Myocastor coypus* Molina, 1782) was found to be naturally infected by *F. hepatica* [6, 8], notably in the populations established in the western part of the territory where the infection rate can be exceedingly high [21]. Nevertheless, the variability of the developmental success of fluke eggs shed by different host species [26] shows that further studies are necessary to consider *M. coypus* as an efficient host of *F. hepatica* and to define its epidemiological role. The purpose of the present study was to explore the role of this rodent as an efficient host of *F. hepatica*. The prevalence of the fluke infection of *M. coypus* in Loire-Atlantique (department of western France) was measured. The developmental success of fluke eggs excreted by *M. coypus* and the infectivity of the parasite to intermediate and definitive host species were experimentally examined.

2. MATERIALS AND METHODS

2.1. Prevalence of *M. coypus* fasciolosis in Loire-Atlantique

M. coypus were sampled in the department of Loire-Atlantique between January and July 1996. In a first trial (a), 438 *M. coypus* were collected in 9 areas representative of the department major humid areas (Fig. 1); these animals were shot by hunters in a population control programme of this noxious animal. In a second trial (b), 80 *M. coypus* were trapped in three farms where *F. hepatica* was present as assessed

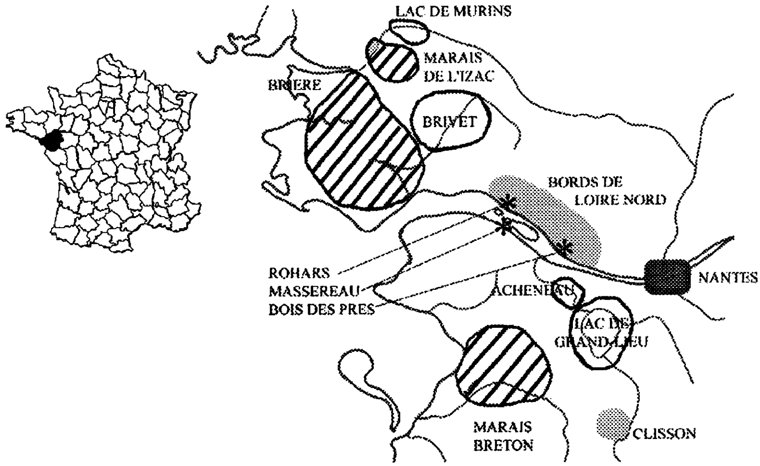

Figure 1. Sampled humid areas and estimation of *M. coypus* infection rates. Shooting campaigns areas with infection rates of 0% (□), 1 to 10 % (▨) and more than 10 % (■). Trapping campaign areas in infected herds (*).

by specific serology in cattle. Additionnal trapping campaigns (c), collecting a total of 224 nutrias, were carried out in one infected area ("Réserve du Massereau") between December 1996 and January 1999.

Animals were sexed and weighed. Bile was extracted by puncture of the gall bladder and fluke eggs were searched in the bile using a sedimentation method. The livers were dissected for recovery and measurement of flukes. Excretion of fluke eggs in faecal samples was measured using a iodomercurate potassium flotation method. *M. coypus* infection rates were calculated by dividing the number of infected *M. coypus* by the number of necropsied *M. coypus* in each sampled area.

2.2. Measure of the infectivity of *M. coypus* liver flukes

2.2.1. Biological materials

Eggs of *F. hepatica* were collected from gall bladders of 43 naturally infected nutrias trapped in the "Réserve du Massereau" (during trial c). Eggs were collected by sedimentation and washed with water; they were incubated for 20 days at 20 °C in total darkness according to the method described by Ollerenshaw [23].

Two populations of *Lymnaea truncatula* snails (Group 1 and Group 2) were collected in the centre of France and used for experimental infection with miracidiae. For some years, these molluscs have been regularly observed for trematode infection and have been known to be free of any trematode infection.

Ten 12-months old male Bellillois sheep were used for experimental infections with metacercariae.

2.2.2. Measure of the hatchability of nutria fluke eggs

Hatching of fluke eggs was provoked by illumination. Forty-three samples of 200 nutria fluke eggs each were used. The hatchability of each sample was measured by counting the empty shells.

2.2.3. Measure of the redial burden and the cercarial shedding [1, 5, 30]

The snails of the two groups were individually exposed for four hours to two *F. hepatica* miracidiae [10] hatched from eggs collected in nutrias (100 snails of each group). The snails were then raised in closed-circuit aquaria for 33 days at 20 °C, with five snails per liter of water.

In the first group, the surviving snails were necropsied at day 33. Quantification of surviving snails, infected snails, total number of rediae and number of rediae per snail, was performed. Snail infection rates were calculated by dividing the number of infected snails by the number of survivors at day 33.

In the second group, the surviving snails were individually placed on day 33 in 35 mm diameter petri dishes with 2-3 mL of water and a piece of lettuce. Daily monitoring consisted in counting metacercariae and changing water until the death of the snail. The dead snails were necropsied to count the rediae. Metacercariae were stored at 4 °C, in the dark, in eppendorf tubes with 0.1 mL of water.

The parameters of cercarial shedding were the number of snails that shed cercariae, the total number of metacercariae, the number of metacercariae shed per snail, the onset and the duration of the patent period, the percentage of floating cysts (calculated in relation to the total number of metacercariae).

2.2.4. Measure of the infectivity in sheep of **F. hepatica** *metacercariae of nutria origin*

Two groups of five sheep were used. Each sheep from the first group was orally infected with 150 metacercariae of *F. hepatica* of nutria origin collected in the previous trial ("measure of the redial burden and the cercarial shedding") and using the method described by Chauvin et al. [7]. Each sheep from the second group was orally infected with 150 metacercariae of a *F. hepatica* isolate cultivated experimentally in sheep for four generations. All animals were bled every two weeks for 12 weeks; blood was centrifuged at 2000 × *g* for 15 minutes. Sera were frozen at – 20 °C until use. Twelve weeks post-infection, all infected sheep were necropsied and flukes were counted. The humoral response was investigated by ELISA with Excretory-Secretory Products of *F. hepatica* (FhESP) as previously described [7].

3. RESULTS

3.1. Measure of the prevalence of *M. coypus* infection in Loire-Atlantique

Among the 742 *M. coypus* autopsied, 160 were found to be infected by liver flukes (Tab. I). The prevalence of the fasciolosis infection of *M. coypus* in Loire-Atlantique (trial a; *n* = 438) was 8.7% (CI 5 %: 6.9 – 10.7). This infection rate varied between 0 and 40 % according to the origin of the animals. In areas where the presence of *F. hepatica* had been shown (trial b, *n* = 80 and trial c, *n* = 224), the prevalence was 40.1 % (CI 5%: 37.0 – 43.3). The infection rate varied between 13 and 57% according to the sampled areas.

In the 742 nutrias, the infection rates in males (86/406; 21.2%) and females (74/336; 22.0%) were similar. The fasciolosis infection rates varied between 0% and 40.5% according to the weight class (Tab. II). Nutrias weighing less than 2 kg were significantly less infected than the other animals.

A total of 915 liver flukes were observed in the 160 infected *M. coypus*. The mean parasitic burden was 5.7 liver flukes per animal with important variations (Fig. 2). Seventy-four percent of the infected animals

Table I. Infection of *M. coypus* with *F. hepatica*, according to 3 trials. a (shooting campaigns 02/96 – 05/96), b (trapping campaigns in infected areas 02/96 – 07/96), c (trapping campaigns 12/96 – 01/99).

	Sampled areas	*Necropsied* M. coypus	*Infected* M. coypus	*Infection rates (%) (CI 5 %)*
Trial a: shooting campaigns 02/96 – 05/96	Acheneau	23	0	0.0 (0.0 – 14.9)
	Bords de Loire Nord	132	32	24.2 (18.8 – 30.3)
	Brière	38	1	2.5 (0.0 – 13.4)
	Brivet	72	0	0.0 (0.0 – 5.0)
	Clisson	5	2	40 (5.4 – 84.9)
	Lac de Grand Lieu	33	0	0.0 (0.0 – 10.6)
	Marais Breton	74	2	2.7 (0.3 – 9.0)
	Marais de l'Izac	32	1	3.0 (0.0 – 15.8)
	Lac de Murins	29	0	0.0 (0.0 – 12.0)
	Total	**438**	**38**	**8.7 (6.9 – 10.7)**
Trial b: trapping campaigns in infected areas 02/96 – 07/96	Bois des prés	28	10	35.7 (20.1 – 53.8)
	Rohars	31	4	12.9 (3.9– 28.4)
	Réserve du Massereau	21	12	57.1 (35.9 – 76.7)
	Total	**80**	**26**	**32.5 (24.3 – 41.4)**
Trial c: trapping campaigns 12/96 – 01/99	Réserve du Massereau	224	96	42.9 (38.8 – 47.0)
Infected areas (b+c)	**Total**	**304**	**122**	**40.1 (37.0 – 43.3)**

Table II. Infection rate of *M. coypus* with *F. hepatica* with regard to body weight.

Weight (kg)	*Observed animals*	*Infected Animals*	*Infection rate (CI) (%)*
≤ 2	**85**	0	0 (0 – 4.3)
2.1 - 3	**135**	24	17.8 (12.9 – 23.5)
3.1 - 4	**159**	30	18.9 (14.4 – 24.0)
4.1 - 5	**140**	38	27.1 (21.8 – 33.0)
5.1 - 6	**149**	38	25.5 (20.4 – 31.1)
> 6	**174**	30	40.5 (31.5 – 50.0)

Animal weighing less than 2 kg were significantly less infected than the others (Fisher's exact test ; $p < 1$ ‰).

had less than 6 flukes. A total of 225 flukes, collected during trials a and b, were measured and the mean fluke length was 20.3 mm; their length varied between 6 and 33 mm (Tab. III) and 65% of the flukes measured more than 18 mm.

3.2. Shedding of faecal fluke eggs

Out of the 144 coproscopic examinations performed, 129 samples showed faecal fluke eggs (90%) and mean fluke egg excretion was 26.9 ± 9.4 eggs per gram (epg); variations were observed between 0 and 386 epg.

3.3. Hatchability of fluke eggs originating from nutrias

The average hatchability rate of fluke eggs collected in nutrias was 39.6%. The hatchability rate varied between 14.0% and 74.6%.

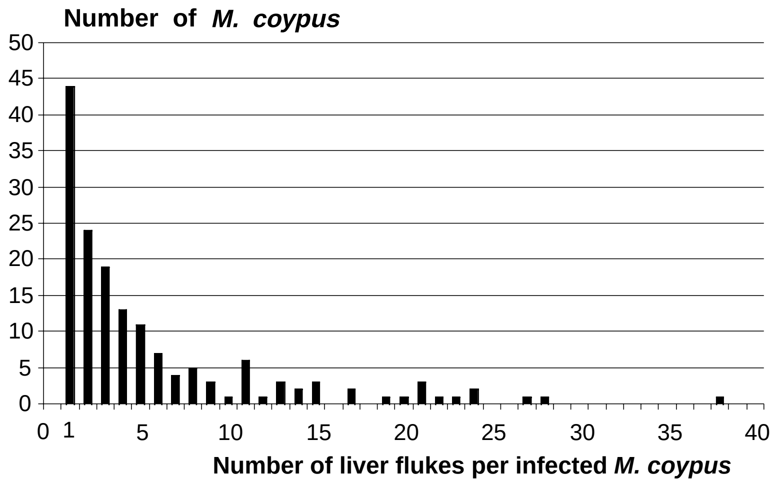

Figure 2. Parasitic burden in infected *M. coypus* (*n* = 160).

3.4. Redial burden and cercarial shedding

The infection characteristics of the two snail groups infected with miracidiae of nutria origin are shown in Table IV. The number of surviving snails was 58 and 73 in the first and in the second snail group, respectively. The proportion of snails with parthenitae was 58.6 and 74.0% in the first and in the second snail group respectively. The mean redial burden was 6.2 in group 1. In group 2, the percentage of snails shedding metacercaria was 27.4% and the number of metacercariae per snail was 72.4 with large variations and a low percentage of floating cysts (5.7%). The onset of the patent period was 54.2 days and the duration of the shedding was short (mean = 9 days). Three shedding waves were observed.

Table III. Length distribution of liver flukes collected from 64 *M. coypus* (*n* = 225, trials a and b).

Length (mm)	*Number of flukes (%)*
> 25	38 (16.9)
18 – 25	109 (48.4)
11 – 17	68 (30.2)
≤ 10	10 (4.4)

3.5. Measure of the infectivity of *F. hepatica* metacercariae of nutria origin for sheep

The mean worm burdens in sheep infected with metacercariae of ovine and nutria origin were 29.6 and 31.6 flukes, respectively. The infection rates and the parasite lengths from the *M. coypus* origin were similar to those of ovine origin (Tab. V). Anti-*F. hepatica* antibodies were detected in the second week post-infection in both groups of sheep (Fig. 3). The antibody level increased rapidly in the two groups, reaching its highest values at the 8–10th week. It decreased slightly afterwards. No difference between the two groups was observed.

Table IV. Characteristics of cercarial shedding in 2 groups of snails infected with miracidiae of nutria origin.

	Group 1	*Group 2*
Number of snails	100	100
– surviving at day 30	58	73
– with live parthenitae	34	54
– with shedding	/	20
Frequency of infected snails (%)	58.6 ± 12.7[a]	74.0 ± 10.0
Frequency of snails with shedding (%)	/	27.4 ± 10.2
Mean number of rediae in infected snails	6.2 ± 1.9	/
Onset of patent period (days)	No data	54.2 +/– 6.2
Duration of patent period (days)	No data	9.0 +/– 7.1
Number of metacercariae per snail	No data	72.4 +/– 52.6
Percentage of floatting cysts	No data	5.7 %
Number of shedding waves	No data	3

[a]: mean ± standard error ($p < 0.05$).

Table V. Experimental infection of sheep with 150 *F. hepatica* metacercariae of nutria or sheep origin.

Infected sheep	*Mean number of flukes*	*Infection rate (%)*	*Mean fluke length (mm)*
Metacercariae from nutria			
071	26	17.3	16.5
073	38	25.3	17.8
076	13	8.7	15.0
077	38	25.3	16.8
082	43	28.7	16.1
Mean	**31.6**	**21.1**	**16.4**
Metacercariae from sheep			
072	40	26.7	15.7
074	55	36.7	16.9
075	12	8.0	12.4
080	15	10.0	12.1
081	26	17.3	13.0
Mean	**29.6**	**19.7**	**14.0**

4. DISCUSSION

Approximately 9% of the *M. coypus* population was naturally infected by *F. hepatica* in the department of Loire-Atlantique. The heterogeneity of the infection rates could be related to the environmental heterogeneity of the studied habitats. Some of these biotopes were unsuitable for the intermediate host *Lymnaea truncatula* [4, 15,

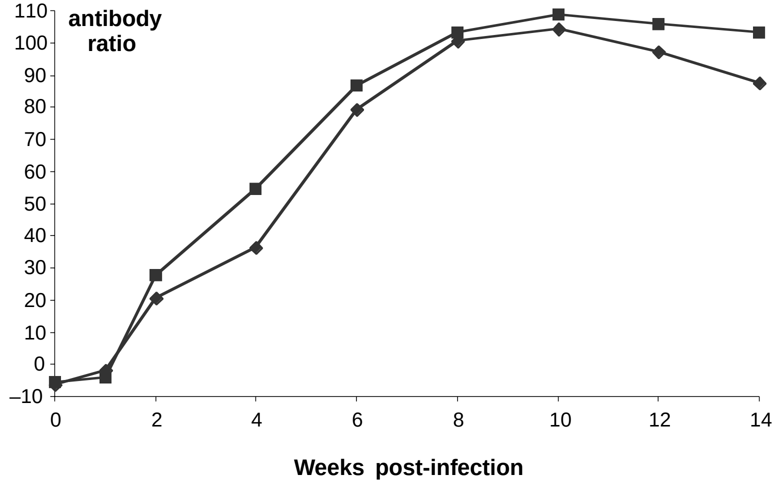

Figure 3. Mean antibody kinetics of sera from sheep infected with *F. hepatica* metacercariae from nutrias (◆ ; *n* = 5) and sheep (■ ; *n* = 5). Results are expressed as an antibody ratio by comparison with laboratory positive and negative standards. Positive threshold is 20%.

18]. The low number of infected nutrias observed in these areas could be explained by the low parasite population. Conversely, in all the areas where the presence of the parasite had previously been established, the populations of nutrias were regularly infected as previously observed by Boussinesq et al. [6]. The structure of *F. hepatica* infrapopulations showed that, in spite of a low parasitic burden, 65% of the liver flukes measure more than 18 mm, which is the size of sexual maturity according to Popescu and Fromunda [24] and the majority of infected nutria shed fluke eggs, with higher faecal egg counts than in infected cattle, with equivalent parasitic burdens [11]. These eggs had lower developmental success than eggs rejected by domestic ruminants [26].

Infection rates measured in the two snail populations (58.6% and 74.0%) and the number of metacercariae per infected snail (72.4) were equivalent to those measured by other authors during experiments on *L. truncatula* infected with miracidiae of rabbit origin but lower than those observed with miracidiae of ovine or bovine origins [9, 26]. These data have to be related to the receptivity of each snail population to *F. hepatica* miracidiae, which depends on many factors, notably the origin of the snail population, the frequency of contacts between snails and *F. hepatica* [25], the origin of the miracidiae… Haroun and Hillyer [12] showed that the nature of the host that carries *F. hepatica* adult form influences the development of the trematode in the intermediate host or in the next final host. In this experiment, the snail infection results could be explained by a misadjustment between the parasite of nutria origin and the snails used, notably because of the use of snails originating from a different area than the fluke isolates.

Metacercariae of nutria origin were infective to sheep and seemed to be as infective as metacercariae of ovine origin. The installation rates were close to those reported in the literature when sheep were infected with metacercariae of sheep origin [7, 32]. This result is different from those obtained during cross-experimental infections between sheep, cattle and rabbits [2, 8, 14, 27].

M. coypus is able to ensure the complete development of *F. hepatica*. The recent introduction of *M. coypus* in France [13] could be at the root of a partial adaptation of *F. hepatica* to this new host and could explain the weak fertility of shed eggs and the poor metacercarial production. The studies initiated by Santos et al. [28], in Brazil, in the original habitat of this rodent should provide further information on this hypothesis. The development of *F. hepatica* in *M. coypus* is more efficient than in rabbits. The epidemiological role of *M. coypus* is compounded by the fact that, by contrast to rabbits, it often defaecates in water [13], and thus sheds fluke eggs close to habitats of potential intermediate host snails, a behaviour very much to the benefit of *F. hepatica* and its life cycle. *M. coypus* is also an opportunistic rodent whose presence was observed in very varied wet biotopes. Its very dense populations [13], its strong capacity of colonising wet areas and its receptivity to *F. hepatica* show that *M. coypus* could be a wild reservoir host of *F. hepatica* in France. *F. hepatica* infection of *M. coypus* (or other wild hosts) should be considered in the establishment of the control programmes of domestic animals and human fasciolosis.

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REFERENCES

[1] Abrous M., Roumieux L., Dreyfuss G., Rondelaud D., Mage C., Proposition d'une technique simple pour la production métacercarienne de *Fasciola hepatica* à partir du mollusque *Lymnaea truncatula* Müller. Rev. Méd. Vét. 149 (1998) 10.

[2] Alcibar P.M., Flores C.R., Evaluation de la infectividad de miracidios y metacercaria de *Fasciola hepatica* en bovinos y ovinos con relacion asu origen. Tec. Pecu. Mex. 48 (1985) 39–45.

[3] Bailenger J., Tribenley J., Amyot B., Duret J., Importance des léporidés comme réservoir sauvage dans l'épidémiologie des distomatoses à *F. hepatica* et *D. dendriticum*, Ann. Parasitol. Hum. Comp. 40 (1965) 51–54.

[4] Baudet J., Gruet Y., Maillard Y., Distribution de certaines espèces de la malacofaune aquatique du Marais-Breton, Haliotis 18 (1988) 21-31.

[5] Boray J.C. Experimental fasciolasis in Australia, Adv. Parasitol. 7 (1969) 96–210.

[6] Boussinesq X., Le Pape P., Ferre J.Y., Marjolet M., La fasciolose en Loire-Atlantique. Prévalence de l'affection chez le Ragondin *M. coypus* : premiers résultats, Bull. Soc. Fr. Parasitol. 4 (1986) 39–43.

[7] Chauvin A., Bouvet G., Boulard C., Humoral and cellular immune responses to *Fasciola hepatica* experimental primary and secondary infection in sheep, Int. J. Parasitol. 25 (1995), 1227–1241.

[8] Delecole J.P., Le Ragondin et la douve, Bull. Soc. Vet. Prat. Fr. 65 (1981) 391–392.

[9] Dreyfuss G., Rondelaud D., *Fasciola hepatica*: a study on the shedding of cercariae from *Lymnaea truncatula* raised under constant conditions of temperature and photoperiod, Parasite 1 (1994) 401-404.

[10] Dreyfuss G., Rondelaud D., *Fasciola gigantica* and *F. hepatica*: a comparative study of some characteristics of *Fasciola* infection in *Lymnaea truncatula* infected by either the two trematodes, Vet. Res. 28 (1997) 123–130.

[11] Euzeby J., Les maladies vermineuses des animaux domestiques et leurs incidences sur la pathologie humaine, Tome II, Section 2, Book 1, Vigot Frères Editeurs, Paris, 1971, 798 p.

[12] Haroun E.M., Hillyer G.V., Resistance to fasciolosis. A review, Vet. Parasitol. 20 (1986) 63–93.

[13] Jouventin P., Micol T., Verheyden C., Guedon G., Le ragondin : biologie et méthodes de limitation des populations, Acta Ed., 1995, p 28–29.

[14] Knight R.A., Experimental cross infection of *Fasciola hepatica* in calves and lambs. J. Parasitol. 64 (1978) 601–605.

[15] Lucas A., Les mollusques aquatiques de la Brière et des eaux environnantes, Bull. Soc. Sci. Nat. Ouest Fr. 64 (1967) 3–13.

[16] Mage C., Conséquences zootechniques de l'infestation naturelle de *Fasciola hepatica* chez des

taurillons limousins, Rev. Méd. Vét. 141 (1990) 205–208.

[17] Mage C., Loisel J., Bonnand P., Infection par *Fasciola hepatica* en élevage laitier, Rev. Méd. Vét. 140 (1989) 929–931.

[18] Maillart Y., Gruet Y., Introduction à l'hydroécologie des marais saumâtres du bassin du Brivet, Actes du 97e Congrès National des Sociétés Savantes, 1972, 276–282.

[19] Mas-Coma S., Fons R., Feliu C., Bargues M.D., Valero M. A., Galan Puchades M.T., Small mammals as natural definitive host of the liver fluke, *Fasciola hepatica* Linnaeus, 1758 (Trematoda: *Fasciolidae*): a review and two new records of epidemiologic interest on the Island of Corsica, Riv Parassitol. 5 (1988) 73–78.

[20] Mas-Coma S., Fons R., Feliu C., Bargues M.D., Valero M.A., Galan Puchades M.T., Conséquences des phénomènes liés à l'insularité dans les maladies parasitaires. La grande douve du foie (*Fasciola hepatica*) et les muridés en Corse, Bull. Soc. Neuchâtel Sci. Nat. 110 (1989) 57–62.

[21] Ménard A., L'Hostis M., Leray G., Marchandeau S., Pascal M., Roudot N., Michel V., Chauvin A., Inventory of wild rodents and lagomorphs as natural hosts of *Fasciola hepatica* on a farm located in a humid area in Loire-Atlantique (France), Parasite 7 (2000) 77–82.

[22] Molan A.L., Hussein N.M.S., A general survey of blood and tissue parasites of some rodents in Arbil province, Iraq, Acta Pathol. Microbiol. Immunol. Scand. 96 (1988) 47–49.

[23] Ollerenshaw C.B., Some observations on the epidemiology of fasciolosis in relation to the timing of molluscicide applications in the control of the disease, Vet. Rec. 98 (1971) 152–164.

[24] Popescu S., Fromunda V., Contribuii la cunoasterea desvoltarii somatice a trematodului *F. hepatica* cu implicatii practice in epizootologia invaziei, Lucr. Inst. Cercet. Vet. Bioprep. Pasteur 12 (1976) 229–235.

[25] Rondelaud D., Variabilité interpopulationnelle de l'infestation fasciolienne chez le mollusque *Lymnaea truncatula* Müller. Influence du contact préalable de la population avec le parasite, Bull. Soc. Zool. Fr. 118 (1993) 185–193.

[26] Rondelaud D., Dreyfuss G., *Fasciola hepatica*: the influence of the definitive host on the characteristics of infection in the snail *Lymnea truncatula*, Parasite 2 (1995) 275–280.

[27] Santiago-Valencia C., Ibarra-Velarde F., Boulard C., Vasquez-Pelaez C., Comparison of the infectivity and antigenicity, in rabbits, of *Fasciola hepatica* metacercariae from bovine, ovine and rabbit origin. II Congreso Nacional de Parasitologia Veterinaria – Veracruz, Argentina, 1992.

[28] Santos, I.C.S., Scaini C.J., Rodrigues L.A.F., Silva Santos I.C., *Myocastor coypus* as a wild reservoir host of *Fasciola hepatica* (Linnaeus, 1758), Rev. Bras. Parasitol. Vet. 1 (1992) 27–30.

[29] Spratt D.M., Presidente P.J.A., Prevalence of *Fasciola hepatica* infection in native mammals in Southeastern Australia, Aust. J. Exp. Biol. Med. Sci. 59 (1977) 713–721.

[30] Taylor E.L., Fascioliasis and the liver fluke in F.A.O, Agricultural Studies 64 (1969) 71–73.

[31] Valero M.A., Marcos M.D., Fons R., Mas-Coma S., *Fasciola hepatica* development in the experimentally infected black rat *Rattus rattus*, Parasitol. Res. 84 (1998) 188–194.

[32] Wedrychowitz H., Turner K., Pfister K., Holmes P.H., Armour J., Local antibody responses in the bile and faeces of sheep infected with *Fasciola hepatica*, Res. Vet. Sci. 37 (1984) 44–51.

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