COCCIDIOSIS OF THE WILD RABBIT (*ORYCTOLAGUS CUNICULUS*) IN FRANCE

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Summary:

In 1998-1999 a survey of coccidiosis in wild rabbits was carried out in six different localities in France. About five individuals were caught monthly in each locality and a total of 254 wild rabbits was examined. Ten species of Coccidia were identified: *Eimeria perforans*, *E. flavescens*, *E. piriformis*, *E. exigua*, *E. media*, *E. magna*, *E. coecicola*, *E. stiedai*, *E. roobroucki*, *E. intestinalis*. Intensity of infection in young individuals was higher than in adults. Intensity was highest in winter but, as there are no young rabbits in winter, in young individuals it was higher in spring and autumn than in summer. Intensities were higher in the northern rather than in southern localities. Ranking of prevalence was remarkably stable, in contrast to the variability of the parasitic load. The equilibrium between congeneric species of rabbit coccidia (stable prevalence rank, variable parasitic load) is thought to be probably the consequence of the opportunistic feeding habits of rabbits

KEY WORDS : *Eimeria*, wild rabbit, congeneric species.

Résumé : LA COCCIDIOSE DU LAPIN DE GARENNE (*ORYCTOLAGUS CUNICULUS*) EN FRANCE

En 1998-1999, nous avons mené une étude sur la coccidiose du lapin de garenne dans six localités différentes en France. Pour chaque site, cinq individus environ ont été capturés mensuellement et un total de 254 lapins a été examiné. Dix espèces de Coccidies ont pu être identifiées : *Eimeria perforans*, *E. flavescens*, *E. piriformis*, *E. exigua*, *E. media*, *E. magna*, *E. coecicola*, *E. stiedai*, *E. roobroucki*, *E. intestinalis*. Chez les jeunes lapins, l'intensité de l'infection apparaît plus élevée que chez les adultes. C'est en hiver que l'intensité de l'infection des adultes est la plus élevée. Par contre, chez les juvéniles, qui n'apparaissent qu'au printemps, l'infection est plus importante au printemps et à l'automne qu'en été. Les sites du nord présentent les intensités les plus élevées. L'ordre des prévalences apparaît remarquablement stable comparé à la variabilité de la charge parasitaire. L'équilibre entre les espèces congénériques de coccidies de lapins (ordre des prévalences stable, charge parasitaire variable) pourrait être la conséquence du comportement alimentaire opportuniste du lapin de garenne.

MOTS CLÉS : *Eimeria*, lapin de garenne, espèces congénériques.

INTRODUCTION

Rabbit *Eimeria* have been extensively used as experimental models, because of the small size of their host, its great fecundity and economic importance. Information on this group of parasites is exceptionally extensive and precise (Coudert *et al.*, 1995), but it mainly concerns the domestic rabbit. The present work is an attempt to compare the fauna of wild and domestic rabbits and to understand the type of balance achieved between the parasite species. Results were compared with reports from the few other countries where similar surveys were performed.

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MATERIALS AND METHODS

STUDY AREAS

The survey was carried out in six study areas.

Arjuzanx reserve covers 2,452 ha in the south-west of France (department of Landes, 44° 00' N, 0° 51' W). The climate is oceanic, the mean annual rainfall is 1,016 mm and the mean annual temperature 12.9° C. Rabbits were caught in mesophilic land colonised by *Ilex europaeus*, *Caluna vulgaris*, *Erica cinerea* and *Erica scoparia*.

Chèvreloup arboretum occupies 200 ha in Versailles near Paris (department of Yvelines, 48° 50' N, 02° 06' E). The climate is oceanic with continental influence, the mean annual rainfall is 606 mm and the mean annual temperature 10.3° C. The terrain is mesophilic grassland.

Donzère-Mondragon reserve covers 1,545 ha in the south-east of France (departments of Drôme and Vaucluse, 44° 26' N, 4° 42' E). The climate is mediterranean with continental influence. The mean annual rain-

fall is 860 mm and the mean annual temperature 12.8°C. It is very diversified xerophilic wasteland supporting Graminaceae, Fabaceae and Compositae and some shrubs, mainly *Cornus sanguinea*, *Populus alba*, *P. nigra*, *Crataegus monogyna* and *Robinia pseudacacia*.

Massereau reserve covers 393 ha in the west of France (department of Loire-Atlantique 47° 14' N, 01° 55' W). The climate is oceanic, the mean annual rainfall is 780 mm and the mean annual temperature 11.7°C. It comprises mesophilic meadowland with mainly *Agrostis* sp., *Oleus lanatus* and *Trifolium* spp., a canal border with *Iris* spp, *Juncus* spp, *Phragmites communis* and a dam bordering a swamp, with *Prunus spinosa* and *Rubus fruticosus*.

Gerstheim reserve has an area of 2,630 ha; it is located in the east of France (department of Bas-Rhin) (48° 22' N, 7° 42' E). The climate is continental, the mean annual rainfall is 650 mm and the mean annual temperature is 10°C. The region used for trapping consisted of three artificial sites with small shrubby vegetation of the mesobrometum or mesoxerobrometum type.

Béniguet island reserve has an area of 64 ha and is located three miles off Conquet in Brittany (48° 21' N, 04° 51' W). The climate is oceanic, the mean annual rainfall is 800 mm and the mean annual temperature is 11.8°C. The terrain comprises xerophilic dunes covered with *Armeria maritima*, *Festuca rubra*, *Senecio jacobaea*, *Silenae matitima* and *Carex arenaria*.

It was not possible to evaluate, in the various reserves, the rabbits density per hectare.

SAMPLING

Five rabbits were usually caught each month from February 1998 to February 1999 in the five mainland areas.

Twenty rabbits were caught on a single occasion in the Béniguet reserve, on the 23 and 24th of September 1998.

Caecal contents were diluted with two volumes of 2.5 % potassium dichromate solution and samples were kept for three days at room temperature of 24°C, in large Petri dishes to allow maturation of the oocysts. The faecal suspensions were then filtered through sieves of decreasing mesh size (400 µm, 200 µm, 100 µm). Oocysts were isolated by magnesium sulphate flotation, identified and counted with the aid of a MacMaster cell. Results are expressed as the number of oocysts per gram of caecal contents. The age of rabbits was evaluated by weighing the crystalline lenses, previously fixed with formaldehyde solution. Rabbits were classified as young (crystalline lenses weighing less than 100 mg) or adult (crystalline lenses weighing more than 100 mg) according to Myers & Gilbert (1968).

Sporulated oocysts were identified according to the published morphological criteria, particularly those of Coudert *et al.* (1995).

RESULTS

Ten species were identified: *Eimeria perforans* (Leuckart, 1879); *E. flavescens* Marotel & Guilhon, 1941; *E. piriformis* Kotlan & Pôspesch 1934; *E. exigua* Yakimoff, 1934; *E. media* Kessel, 1929; *E. magna* Pérard, 1925; *E. intestinalis* Cheissin, 1948; *E. stiedai* (Lindemann, 1865); *E. coecicola* Cheissin, 1947 and *E. roobroucki* described recently by Grès, Marchandeau & Landau (2002). No specific hare coccidia were identified.

Detailed figures of prevalence and parasite load have been recorded in a book (LV) deposited in our laboratory (Parasitologie comparée et modèles expérimentaux) at the Muséum National d'Histoire Naturelle in Paris.

The sex of the animals had no impact on infections which varied with the age of the rabbits, the season and the locality.

Tables I to IV and figures 1 to 4 summarize our results. It was not possible to publish here all the detailed

	Arjuzanx (n = 44)	Béniguet (n = 19)	Chèvreloup (n = 19)	Donzère-M. (n = 53)	Massereau (n = 39)	Gerstheim (n = 43)
<i>E. perforans</i>	923 ± 545	189 ± 73	11,499 ± 5,449	228 ± 52	596 ± 123	2,069 ± 733
<i>E. flavescens</i>	276 ± 59	221 ± 62	4,583 ± 2,246	193 ± 39	327 ± 70	1,183 ± 485
<i>E. media</i>	125 ± 72	270 ± 146	2,897 ± 1,423	40 ± 14	733 ± 433	1,490 ± 1,106
<i>E. exigua</i>	8 ± 2	3 ± 2	1,937 ± 1,121	5 ± 3	39 ± 14	585 ± 544
<i>E. piriformis</i>	70 ± 20	170 ± 138	5,596 ± 2,817	67 ± 234	56 ± 20	77 ± 25
<i>E. stiedai</i>	10 ± 10	7 ± 5	54 ± 40	0.4 ± 0.4	5 ± 4	6 ± 6
<i>E. magna</i>	1 ± 0.3	15 ± 7	84 ± 65	2 ± 1	5 ± 3	10 ± 5
<i>E. coecicola</i>	17 ± 14	0.4 ± 0.4	665 ± 486	11 ± 6	18 ± 15	110 ± 54
<i>E. intestinalis</i>	0	38 ± 34	3,151 ± 2,842	0	0.1 ± 0.1	13 ± 13
<i>E. roobroucki</i>	2 ± 1	0.7 ± 0.4	0	0.1 ± 0.1	2 ± 2	1 ± 1

Table I. – Mean parasitic load of adult rabbits according to locality (± standard deviation).

	Arjuzanx (n = 9)	Béniguet (n = 1)	Chèvreleoup (n = 9)	Donzère-M. (n = 7)	Massereau (n = 1)	Gerstheim (n = 10)
<i>E. perforans</i>	16,840 ± 7,415	164	167,545 ± 80,793	13,763 ± 10,370	4,453	107,236 ± 69,381
<i>E. flavescens</i>	4,529 ± 2,390	15	76,533 ± 43,807	3,021 ± 1,269	4,593	43,372 ± 41,738
<i>E. media</i>	19,935 ± 13,566	0	19,885 ± 16,689	1,350 ± 1,071	0	49,923 ± 28,414
<i>E. exigua</i>	874 ± 652	0	1,988 ± 1,196	285 ± 179	0	2,512 ± 2,139
<i>E. piriformis</i>	4 ± 4	140	11,121 ± 6,399	234 ± 106	73	0
<i>E. stiedai</i>	9,575 ± 9,408	0	72,112 ± 50,966	14,025 ± 12,621	347	45,768 ± 27,327
<i>E. magna</i>	1,785 ± 1,566	6	52,014 ± 51,483	975 ± 780	120	20,995 ± 13,265
<i>E. coecicola</i>	38 ± 38	0	38,941 ± 25,536	53 ± 53	193	32,933 ± 16,055
<i>E. intestinalis</i>	0	0	16,732 ± 10,810	0	0	0
<i>E. roobroucki</i>	0	0	0	0	0	0

Table II. – Mean parasitic load of juvenile rabbits, according to locality (± standard deviation).

	Spring (n = 52)	Summer (n = 40)	Autumn (n = 67)	Winter (n = 58)
<i>E. perforans</i>	783 ± 189	493 ± 230	1,109 ± 534	4,349 ± 1,890
<i>E. flavescens</i>	293 ± 48	1,333 ± 986	775 ± 267	979 ± 436
<i>E. media</i>	526 ± 323	139 ± 97	603 ± 301	1,503 ± 889
<i>E. exigua</i>	48 ± 19	26 ± 14	417 ± 353	562 ± 377
<i>E. piriformis</i>	102 ± 37	249 ± 183	609 ± 369	1,132 ± 880
<i>E. stiedai</i>	1 ± 0.6	19 ± 13	19 ± 15	5 ± 5
<i>E. magna</i>	5 ± 3	2 ± 1	10 ± 4	28 ± 22
<i>E. coecicola</i>	22 ± 13	70 ± 40	46 ± 37	213 ± 159
<i>E. intestinalis</i>	0	0	106 ± 84	932 ± 932
<i>E. roobroucki</i>	3 ± 2	0	0.2 ± 0.1	0.4 ± 0.2

Table III. – Mean parasitic load of adult rabbits according to season (± standard deviation).

	Spring (n = 9)	Summer (n = 18)	Autumn (n = 9)
<i>E. perforans</i>	68,597 ± 27,730	56,232 ± 37,738	133,554 ± 85,980
<i>E. flavescens</i>	52,333 ± 44,422	11,118 ± 8,026	57,250 ± 45,502
<i>E. media</i>	50,211 ± 28,617	3,239 ± 1,906	39,447 ± 23,868
<i>E. exigua</i>	4,124 ± 2,449	448 ± 332	833 ± 579
<i>E. piriformis</i>	1,796 ± 1,726	4,075 ± 3,368	1,353 ± 658
<i>E. stiedai</i>	92,789 ± 51,533	4,983 ± 4,698	40,518 ± 25,431
<i>E. magna</i>	8,628 ± 8,224	34,442 ± 26,120	385 ± 196
<i>E. coecicola</i>	0	18,120 ± 9,407	39,532 ± 25,744
<i>E. intestinalis</i>	0	5,035 ± 5,035	6,663 ± 5,677
<i>E. roobroucki</i>	0	0	0

Table IV. – Mean parasitic load of juvenile rabbits according to season (± standard deviation).

tables and graphs. They may however be found in V. Grès thesis (2001):

- the mean parasite load of adult (Table I) and young (Table II) rabbits from each locality (for all seasons together);
- the mean parasite load of adult (Table III) and young (Table IV) rabbits at different seasons;
- prevalences of the different species in each study area throughout the year, in adult (Fig. 1) and young (Fig. 2) rabbits; and
- prevalences of the different species in adult (Fig. 3) and young (Fig. 4) rabbits according to the season. There were no young rabbits present in winter.

INTENSITY OF INFECTION

Parasite load was variable in young rabbits, possibly depending on the uncertain chance of their becoming infected.

In adult rabbits, intensity of infection was generally highest in winter. In young animals it was higher in spring and autumn than in summer except for *E. piriformis* and *E. magna*.

In adult rabbits, the intensity of infection was generally highest in winter.

The principal feature determining the intensity of infection was the geographical location. Parasite load was

Fig. 1. – Prevalences of the *Eimeria* species in adult rabbits, according to locality.

Fig. 2. – Prevalences of the *Eimeria* species in juvenile rabbits, according to locality.

Fig. 3. – Prevalences of *Eimeria* species in adult rabbits, according to season.

Fig. 4. – Prevalences of *Eimeria* species in juvenile rabbits, according to season.

high in the humid and relatively cold localities (Ile de France and Alsace), lower along the Atlantic littoral (Loire and Landes) and lower still in the dryer and warmer areas (Rhône valley).

PREVALENCES

In adult rabbits, prevalence was highest with two species, *E. perforans* and *E. flavescens*; it was moderately high with *E. media* and *E. piriformis*, and generally low with the six other species.

Results from young rabbits were available (Figs 2 and 4) from only four locations (seven to 10 rabbits examined from each): Arjuzanx, Chèvreloup, Donzère-Mondragon and Gersheim. *E. roobroucki* was absent and *E. intestinalis* was found only in Chèvreloup.

Prevalences were, with few exceptions, remarkably similar in the different localities: nearly 100 % with *E. perforans* and *E. flavescens* and 70 % with the six other species. Two exceptions were *E. piriformis*, absent from Gersheim, and *E. exigua* and *E. coecicola*, which had low prevalences (around 10-30 %) in Arjuzanx and Donzère-Mondragon.

The histogram of prevalence (Fig. 4) according to season shows least variability in autumn compared with spring and summer, i.e. *E. perforans* and *E. flavescens* around 100 % and the six other species around 70 %.

With the adult rabbits, in contrast with the variability of parasite load according to location (Fig. 1), we observed stable prevalences: *E. perforans* and *E. flavescens*, 90-100 %; *E. piriformis*, 70-95 %; *E. exigua*, 32-72 %; *E. media*, 29-69 %; *E. magna*, 17-42 %; *E. coecicola*, 5-32 %; *E. stiedai*, 4-21 %; *E. roobroucki*, 0-21 %; and *E. intestinalis*, 0-16 %. Even more striking, in all localities, the ranked order of prevalence of each species was remarkably stable: *E. perforans* and *E. flavescens*, 1-2; *E. piriformis*, 3; *E. exigua*, 4-5 (with low parasite loads); *E. media*, 4-5; *E. magna*, 6; *E. coecicola* and *E. stiedai*, 7-9; *E. roobroucki* and *E. intestinalis*, 9-10.

DISCUSSION

COMPARISON OF COCCIDIOSIS OF WILD AND DOMESTIC RABBITS

For this type of study, precise specific identification is necessary but the systematics of Coccidia are difficult. Congeneric species are frequent with the Nematodes, for example, and their morphology is varied with many differential characters, while with *Eimeria* the morphology of the oocyst, which is the main criterion is a weak character. Confusion between species is always possible and it is disturbing to find in the literature different biological characters

assigned to the same species by reliable authors. In the same way the origin of strains is not always specified, and biological differences observed amongst strains may in fact represent subspeciation.

The parasitism of domestic rabbits differs from that in the wild: Zundel (1979) carried out a survey in breeding colonies in different parts of France and listed nine intestinal species. Two of the species recorded, although designated by different names, correspond to species found by us in the wild: *Eimeria* sp. sensu Zundel = *E. flavescens*, and *E. neoleporis* sensu Zundel = *E. coecicola*. His list differs from ours by the presence of *E. irresidua* and the absence of *E. exigua* and *E. roobroucki*. The percentages of prevalence (P) and parasite load (PL) were approximately as follows: *E. perforans*: P 87 %, PL 37 %; *E. media*: P 55 %, PL 10 %; *E. magna*: P 53 %, PL 37 %; *E. flavescens*: P 30 %, PL 1 %; *E. irresidua*: P 25 %, PL 1 %; *E. intestinalis*: P 21 %, PL 10 %; *E. piriformis*: P 13 %, PL 1 %; and *E. coecicola*: P 11 %, PL 10 %. However, contrary to what we observed with wild rabbits, the ranking of prevalence varied from one region to the other.

The predominance in breeding colonies of domestic rabbits of the three species *E. perforans*, *E. media* and *E. magna* has also been reported in Italy (Gallazzi, 1977), England (Catchpole & Norton, 1979), Belgium (Peeters & Geeroms, 1981), Saudi Arabia (Kasim & Al-Shawa, 1987), Nigeria (Okewole, 1990), and India (Souza *et al.*, 1993). These species remained dominant in the industrial breeding colonies after developing resistance to robenidine.

In contrast to these observations, in the wild rabbit in its natural environment, in south-west Australia, Hobbs & Twigg (1998) found prevalences to be very similar to ours in France: *E. perforans*, 84 %; *E. flavescens*, 60 %; *E. piriformis*, 50 %; *E. exigua*, 62 %; *E. media*, 45 %; *E. magna*, 33 %; *E. coecicola*, 7 %; *E. stiedai*, 26 %; and *E. intestinalis*, 19 %. The only important difference was the presence in Australia of *E. irresidua* (9 %) and the absence of *E. roobroucki*. This was not true, however, for the parasite intensities.

THE BALANCE BETWEEN CONGENERIC SPECIES

Several factors have influenced speciation of *Eimeria* in rabbits: a long evolutionary period, the Lagomorpha having evolved little since the Eocene and having retained their parasitic fauna, and geographical isolation together with monoxenous life cycles and oocysts which appear to be (Coudert & Ivoré, 1973) fragile and destroyed by heat.

Rabbits are strictly herbivorous, but they are opportunistic feeders which accounts for the variability of parasite intensity from each individual. However the stability of the rank of prevalence indicates an ancient and highly stable equilibrium.

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