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PRESENCE OF INFECTIOUS AGENTS AND PARASITES IN WILD POPULATION OF COTTONTAIL (*Sylvilagus floridanus*) AND CONSIDERATION ON ITS ROLE IN THE DIFFUSION OF PATHOGENS INFECTING HARES

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Abstract

In three study areas located in Alessandria province (Piedmont, Italy), 271 *Sylvilagus floridanus* were captured for parasitological and serological survey. The parasitological research has shown the presence of ecto- (ticks, fleas) and endoparasites (nematodes, protozoa), and in particular the infestation with exotic species (*Euhoplopsyllus glacialis*, *Obeliscoides cuniculi*, *Trichostrongylus calcaratus*, *Passalurus nonannulatus*). Sera were tested to detect antibodies against the following diseases: EHBS and RHD, myxomatosis, leptospirosis, brucellosis, tularemia, Lyme borreliosis, toxoplasmosis and encephalitozoönosis. Experimental infection of 6 cottontails and 6 domestic rabbits (all sero-negatives for RDHV and EBHSV) with virulent RDHV strain, induced mortality only in domestic rabbits. Experimental infection of 4 cottontails and 2 hares (all sero-negatives for RDHV and EBHSV) with virulent EBHSV strain, induced mortality in a cottontail. The results and their sanitary aspects are discussed.

Key words: cottontail rabbit, *Sylvilagus floridanus*, infectious diseases, parasitological diseases.

Introduction

The eastern cottontail (*Sylvilagus floridanus*), a native American Lagomorpha, was introduced in different European countries: France (1953), Italy (1966), Spain (1980), Switzerland (1982). The largest "Italian" population lives in Piedmont, North Italy, where the eastern cottontail is felt as a sort of pest for agriculture and a biological competitor of the brown hare (*Lepus europaeus*) (17). Since few data are available on the diffusion of infectious agents and parasitic agents of this species in our country and in Europe we carried out a seroepidemiological survey and parasitological analysis with the aim to determine their role as hosts, vehicles or reservoirs of pathogens common to hares.

Area of study

We considered three different areas in the province of Alessandria (lat. 44,916; long. 8,6148), Italy, with the following criteria: i) typical habitat of all territories occupied by *S. floridanus*; ii) high density of *S. floridanus* (>100 animals/Km^2); iii) contemporary presence of brown hares; iv) no hunting allowed.

Materials and methods

Fifteen individuals were shot in each study area at bimonthly intervals, between July 1999 and August 2000. A total number of 271 animals (131 males, 140 females) were collected. Each animal was submitted to both serological and parasitological analyses.

Parasitological analyses
Detection and identification of: ectoparasites in animals' fur, protozoa in faeces, helminths in the digestive tract, nematodes in the respiratory system and trematodes in the liver.
In each area, for each species of nematodes we determined the prevalence (P), the relative abundance (RA) and the abundance (A).

Serological examination
The serum was analysed to detect antibodies to the following infections using the reported tests and the relative "cut-off" indicated in brackets:

- Rabbit Haemorrhagic Disease (RHD): Competitive ELISA (≥1/10)
- European Brown Hare Syndrome (EBHS): Competitive ELISA (≥1/10)
- Myxomatosis: Competitive ELISA (≥1/10)
- Leptospirosis (8 serovars of *Leptospira interrogans*): MicroAgglutination Test-MAT (≥1/100)
- Brucellosis (*B. abortus melitensis*): Complement Fixation Test-CFT (≥20 U.I.)
- Tularemia: MicroAgglutination Test-MAT (≥1/40)
- Lyme borreliosis: Indirect Immune Fluorescence Test-IFAT (≥1/80)
- Toxoplasmosis: Latex Agglutination Test-LAT (≥1/32)
- Encephalitozoönosis (*Encephalitozoön cuniculi*): Carbon Immuno Assay-CIA (≥1/40).

Experimental trials
We checked the reproducibility of RHD and EBHS in seronegative cottontails under experimental conditions, by inoculation of RHDV and EBHSV virulent strains. In the first trial 2 cottontails and 3 rabbits were infected with the RHDV reference strain BS89 by oro-nasal route, 2 cottontails and 2 rabbits were administered by the same route and inoculum but inactivated, the remaining 2 cottontails and 2 rabbits were maintained as non infected controls. The animals were kept in three separated rooms, checked daily for two weeks and serologically controlled, for RHDV IgM and IgG, after 14 and 28 days post infection (p.i.). In the second experiment 4 seronegative cottontails, and 2 seronegative hares were infected by oro-nasal route with a virulent suspension of EBHSV. The animals were kept in separated cages in the same room, checked daily for two weeks and serologically controlled for EBHSV IgM and IgG after 11 and 32 days p.i..

Results and discussion

Parasites
Three nematodes have been collected from the digestive tract: *Obeliscoides cuniculi*, *Trichostrongylus calcaratus* and *Passalurus nonannulatus.* Their prevalence and abundance are shown in Table 1. Moreover we have found two different genera of cestodes whose identification is in progress.
Seven different *Eimeria* were found: *E. honessi, E. paulistana, E. environ, E. magna, E. leporis, E. andrewsi* (15).
As regards ectoparasites, we have found ticks (*Ixodes ricinus*) and three different fleas: *Ctenocephalides canis*, *Euhoplopsyllus glacialis* and *Ctenophtalmus agyrtes.* Their prevalence is reported in Table 2. No lung or liver parasite was found. Tab. 1 and 2
The nematodes found are first reports in Italy. Rabbits (*Oryctolagus cuniculus*) infected by *O. cuniculi* have been reported in India and China, while in North America the parasite has been found in *S. floridanus*, *Lepus americanus* and *Lepus capensis* (9; 7; 1; 10; 6; 11; 12; 13). *T. calcaratus* has been reported in North America in *S. floridanus*, *L. americanus*, *Sciurus niger* and *Odocoileus virginianus* (16; 2; 9; 4; 3). *P. nonannulatus* has been reported in North America in *S. floridanus* (2).
The flea *E. glacialis* is another first report in Italy. It has been previously reported in North America in *Sylvilagus audubonii, Lepus californicus, L. americanus* and *Urocyon cinereoargenteus* (8; 14; 5).
Our results point out that the importation of an allochthonous species implied the introduction of new parasites in Italy. This might have unpredictable consequences on the sympatric populations of autochthonous lagomorphs. Recently, we have collected *O. cuniculi* and *E. glacialis* in hares come from two of our three study areas (in prep.).

Table 1 – Gastrointestinal nematodes in *S.floridanus*: prevalence (P), relative abundance (RA) and abundance (A) in the three study area.

Area of study	"Roleto"					"Tollara"					"Sezzadio"				
	P	RA	A	min	max	P	RA	A	min	max	P	RA	A	min	max
O. cuniculi	93	18,0	77,5	1	430	84	16,3	52,7	1	770	5	0,7	23,4	1	90
T. calcaratus	90	52,4	234,2	3	1778	88	83,7	287	3	2550	89	99,3	194	1	1560
P. nonannulatus	62	29,7	191,9	1	1920	0					0				

Table 2 – Prevalence of ectoparasites in the three study areas

Area of study	"Roleto"	"Tollara"	"Sezzadio"
I. ricinus	8,8	6,6	5,5
Euhoplopsyllus glacialis	73,3	8,8	96,7
Ctenocephalides canis	1,1	1,1	1,1
Chtenophtalmus agyrtes	0	2,2	1,1

Table 3 - Results of the seroepidemiological investigation (bacterial diseases). Legenda: *1:40, ^1:80, °1=1:64, 2=1:128, 2=1:256 "1:6400 serovar Australis Bratislava

Area of study	*F.tularensis*	*B.burgdorferi*	*T.gondiii*	*L. interrogans*
Roleto	0/83	3/83^	2/81°	0/83
Tollara	0/87	2/87^	2/84°	0/87
Sezzadio	1/83*	1/83^	1/82°	1/83"
Total	*1/253*	*6/253*	*5/247*	*1/253*
Prevalence	*0,4%*	*2,4%*	*2,0%*	*0,4%*

Table 4. Results of the seroepidemiological investigation (viral diseases)

Area of study	Mixomatosis	EBHS	RHDV
Roleto	41/82	8/82	27/82
Bormida	28/83	16/83	25/83
Tollara	44/88	21/87	33/87
Totale	*113/253*	*45/252*	*85/252*
Prevalence	*44,6*	*17,8*	*33,7*

Serology

The positive results are summarised in the table 3. No antibodies were detected against *Brucella* spp. and *E. cuniculi*. Indeed, the results obtained induce to exclude a relevant role of *Sylvilagus* in the dissemination and transmission to hare and other mammals, including humans, of infectious agents such as *F. tularensis, T. gondii, Brucella spp., L. interrogans, E. cuniculi, B.burgdorferi*
Tab. 3 e 4
More interesting are the results concerning the three main viral diseases of lagomorphs (Table 4). The high prevalence for myxomatosis in the absence of known clinical cases (some sera had titres as high as 1/1280) confirms that cottontail that *Sylvilagus* could be infected by this virus. It probably acts as wild reservoir and could play an active role in the epidemiology of myxomatosis of this viral infection in the wild.
Since RHD and EBHS are both considered host-specific diseases, it is more difficult to explain the serological results detected i.e. an overall seroprevalence of 17.8% for EBSV and 33.3% for RHDV antibodies. Indeed, a relevant number of sera had high titres for EBHSV (up to 1/1280), whilst the highest RHDV titre in a single serum was 1/80. These results, compared to those detected in the sera of RHD recovering-convalescent rabbits and EBHS-convalescent hares, indicate that some of

the cottontails could have been naturally infected by the EBHSV, developing a strong immunity. Conversely, the titres for RHDV antibodies were always too low to be considered specific or directly induced by one of the two known calicivirus of lagomorphs.

Experimental trials

The inoculation of RHDV caused an acute form of RHD only in the three seronegative rabbits, which all died within 72-hrs p.i. Showing typical signs and lesions. The RHDV positively was confirmed by sandwich ELISA test. All the remaining animals remained healthy during the 14 days of observation and in their sera, collected at 14 and 28 days p.i., no antibodies (IgM and IgG) were detected using the RHDV ELISAs. These data confirm that cottontails are not susceptible to experimental infection with RHDV, as previously postulated, but also that the inactivated RHDV do not stimulate the immune system of rabbits when administered by oro-nasal as well as it did when injected intramuscularly. During the second trial one cottontail died at 4 days p.i. and at necropsy it had typical gross lesions referable to EBHS. The diagnosis was confirmed by virological ELISA, and by the relevant seroconversion (IgM up to 1/40000 and IgG up to 1/1280) detected in the sera of the hare and the three cottontails that survived. These results demonstrate transmissibility of EBHSV to *S. floridanus* and support the conclusions of the sero-epidemiological survey. In particular, the cottontail could play a role in the spreading of EBHS.

Further epidemiological studies will be pointed to contemporarily detect the incidence of EBHS in sympatric and even syntopic populations of hares and *Sylvilagus* as well as to determine the characteristics (morbidity, lethality, lesions etc) of such disease in *Sylvilagus,* since it could be less severe or totally silent.

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