European brown hare as a potential source of zoonotic agents

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ABSTRACT: There has recently been a growing interest in checking the state of health of European brown hares in hunting grounds because they are a susceptible bio-indicator of environmental changes and because of the dramatic decline in populations across Europe. A total of 1 051 (384, 302, and 365, respectively) blood sera were collected from hares during autumn hunting events and examined for tularaemia, brucellosis and leptospirosis in 2004, 2005 and 2006. Antibodies were found in 6.5, 1.6 and 7.5% of cases, respectively. Antibodies against *Francisella tularensis*, *Brucella suis* and leptospires were found in 7.9, 1.7 and 7.7% of females and 4.5, 1.4 and 7.9% of males, respectively. Higher seroprevalence of tularaemia was found in females ($P = 0.05$). Brucellosis was more prevalent in adult animals compared to subadult ones ($P = 0.05$). Only the *L. grippotyphosa* serotype was found and the titres mostly ranged from 100 to 400 (89.9%). Higher titres from 800 to 3 200 were found in the remaining 10.1% of the examined European brown hare sera. Antibodies against *F. tularensis* as well as *B. suis* were most frequent in low titres of 10 to 80. Higher titres were only exceptional. Confirming that hares are susceptible to various zoonotic agents, it is necessary to be careful when handling the animals killed. On the other hand, blood sera from hares may be used to survey the occurrence of natural nidi of zoonoses in hunting grounds.

Keywords: *Lepus europaeus*; zoonosis; leptospirosis; tularaemia; brucellosis; antibodies

Regarding the production of venison as well as the export of live animals, the European brown hare (*Lepus europaeus*, Pall. 1778) was one of the most important game animals with annual bag records amounting to 1.4 million in the Czech Republic in 1971. It is found in more than 80% of hunting grounds in the Czech Republic and it is most abundant in such regions as the Elbe valley and South Moravia. The population density of the European brown hare is ever changing in dependence on external factors such as abiotic ones (e.g. climate), biotic ones (feed availability, diseases, predators, etc.), anthropogenic ones and internal factors (reproductive rate, ability for adaptation, etc.). There has, however, been documented a population decline of hares during the last three decades in Europe whereas its causes are not fully understood. It is generally supposed that they are polyfactorial. Analysing the mortality rate of 2 269 wild European brown hares in the Czech Republic, Sterba (1982) found that dietetic, parasitic, infectious, toxic and traumatic causes were responsible for 25, 25, 30, 10 and 10% of cases, respectively. According to Haerer et al. (2001) infections were responsible for mortality in 15% of hares. Infectious and parasitic diseases are undoubtedly important biotic factors influencing the population of the hare. The calicivirus causing European brown hare syndrome has resulted in high mortalities and is attributed with the dramatic decline in populations across Europe (Gavier-Widen and Morner, 1991; Duff et al., 1994; Frolich et al., 1996). Apart from common diseases such as staphylococcosis, pasteurellosis, pseudotuberculosis and brucellosis, the European brown hare may harbour many zoonotic agents, of which tularaemia, listeriosis, toxoplasmosis, lept-

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ospirosis and borreliosis are the most serious and dangerous under conditions of careless handling of affected animals (Giraudo et al., 1985; Morner et al., 1988; Frolich et al., 2003; Bartling et al., 2004). Hunters are known to be high in the seroprevalence against some zoonoses due to the high exposure rate (Deutz et al., 2003).

Recently, there has been a growing interest in checking the state of health of hares in hunting grounds because they are a susceptible bioindicator reflecting the action of negative environmental factors. The epizootiological importance of the European brown hare is based on the fact that it is a species maintaining the so-called home range (Kunst et al., 2001), being thus prone to many infectious diseases of natural nidality (Pikula et al., 2003, 2004, 2005). As a part of the monitoring of tularaemia occurrence in the European brown hare in South Moravia, we collected blood sera from shot specimens and examined them for the presence of antibodies against *Francisella tularensis*, *Brucella suis* and leptospires in 2004, 2005 and 2006.

MATERIAL AND METHODS

Blood was collected by a heart puncture from European brown hares shot in the area of long-term surveillance for tularaemia (South Moravia, Czech Republic) in 2004 to 2006. A total of 1 051 (384, 302 and 365, respectively) blood samples were examined during this period. Veterinarians present on each hunting event examined the animals shot and determined their gender and age. Hares were classified as subadult and adult by checking the presence and/or absence of a distal epiphyseal knob in the ulna and radius as described by Stroh (1931). None of the animals was suspected of having been infected by the infectious agents studied. Blood was centrifuged after collection and the resulting serum was deep frozen until further processing. The examination for the presence of antibodies against *F. tularensis* and *B. suis* was performed in a standard way using slow agglutination and commercially available antigens (Bioveta a.s., Ivanovice na Hane). A total of 10 leptospiral strains (*L. grippotyphosa, L. icterohaemorrhagiae, L. sejroe, L. canicola, L. jež bratislava, L. pomona, L. sorex jalna, L. bulgarica, L. arboreae, L. bataviae*) were used to test the presence of antibodies against leptospires using the agglutination lysis reaction. Sera reacting in the standard dilution of 1:100 were examined with the respective serotype up to the titre. Differences in the prevalence of seropositive cases for the three infections studied between males and females, adult and subadult animals as well as groups of hares of individual years were evaluated using the *t*-test for comparing paired relative values.

RESULTS

Tables 1 to 4 document the results concerning seropositive cases of European brown hares for the infectious agents studied. A total of 1 051 blood sera examinations yielded 69 (6.5%), 17 (1.6%) and 79 (7.5%) seropositive cases for tularaemia, brucellosis and leptospirosis, respectively. Considering the seroprevalence of the above infections, there were no statistically significant differences between the individual years of study despite, for example, a decreasing tendency in the percentage of positive cases for brucellosis (Table 1). Table 2 shows the presence of antibodies against selected zoonotic agents with regard to the gender and age of examined animals. A total of 635 females and 416 males were examined. Antibodies against *F. tularensis*, *B. suis* and leptospires were found in 7.9, 1.7 and

Table 1. Annual and total results of serological examinations of the European brown hare for the presence of antibodies against selected zoonotic agents

Year of study	Numbers of examined hares	Antibodies against					
		Francisella		*Brucella*		*Leptospira*	
		n	%	*n*	%	*n*	%
2004	384	23	5.9	11	2.8	30	7.8
2005	302	21	6.9	4	1.3	14	4.6
2006	365	25	6.8	2	0.6	35	9.5
Total	1 051	69	6.5	17	1.6	79	7.5

Table 2. Results of positive findings of antibodies against selected zoonotic agents with regard to the gender and age of examined animals

Numbers of examined hares		Antibodies against					
		Francisella		*Brucella*		*Leptospira*	
		n	%	*n*	%	*n*	%
Females	635	50	7.9	11	1.7	46	7.7
subadult	238	13	5.4	2	0.8	18	7.5
adult	397	37	9.3	9	2.2	28	7.0
Males	416	19	4.5	6	1.4	33	7.9
subadult	165	7	4.2	0	0	13	7.8
adult	251	11	4.3	6	2.3	20	7.9

7.7% of females and 4.5, 1.4 and 7.9% of males, respectively. Considering tularaemia, higher seroprevalence was found in females (P = 0.05). Brucellosis, on the other hand, was more prevalent in adult animals compared to subadult ones (P = 0.05). A total of 10 strains of leptospires were used for testing, however, only the *L. grippotyphosa* serotype was found (Table 3) and the titres mostly ranged from 100 to 400 (89.9%). Higher titres from 800 to 3 200 were found in the remaining 10.1% of the examined European brown hare sera. As shown in Table 4, antibodies against *F. tularensis* as well as *B. suis* were most frequent in low titres of 10 to 80. Higher titres were only exceptional.

DISCUSSION

European brown hares (*Lepus europaeus*) from South Moravia were found seropositive for selected zoonoses such as tularaemia, brucellosis and leptospirosis. These results document the presence of

Table 3. Titres against leptospires found in the European brown hare specimens

Leptospiral serotypes	Total	Titres					
		100	200	400	800	1 600	3 200
L. grippotyphosa							
number of cases	79	48	14	9	3	3	2
percentage	100	60.76	17.71	11.40	3.8	3.8	2.53

Table 4. Titres of antibodies against *Francisella tularensis* and *Brucella suis* in the European brown hare. As shown, low titres of antibodies (10 to 80) against both infectious agents were found most frequently while high titres were only exceptional

Antibodies against	Total	Titres						
		10	20	40	80	160	320	640
Francisella								
number of cases	69	20	9	12	13	10	5	0
percentage	6.5	28.8	13.1	17.4	18.8	14.6	7.3	0
Brucella								
number of cases	17	7	2	2	2	1	1	2
percentage	1.6	42.0	11.6	11.6	11.6	5.8	5.8	11.6

the above pathogens in the environment of the studied area and the susceptibility of the European brown hare to these infections. As documented in our previous studies (Treml et al., 1997), the presence of the above zoonotic infectious agents in the environment may influence the population density of the European brown hare in a negative way. For example, after the epizootics of tularaemia in South Moravia, hunting bags were lower by two thirds in 1995. Haerer et al. (2001) had a similar experience in Switzerland, where mortality due to infections such as brucellosis and tularaemia reportedly amounted to 15%. Another interesting serological study of European brown hares was performed in Schleswig-Holstein by Frolich et al. (2003). These authors also confirmed antibodies against various infectious agents excepting, however, tularaemia and brucellosis.

European brown hares examined in this study came from the area of long-term surveillance of tularaemia which is considered a serious zoonosis with hares playing an important role as a source of infection for humans handling diseased individuals. As already published, the incidence of tularaemia in this area increased considerably in 1994 (Treml et al., 1997) and remained above the long-term average (Treml et al., 2001). Many authors consider the European brown hare highly susceptible to the causative agent of tularaemia (Olsufjev and Rudnev, 1960; Libich, 1981). Even extremely low infectious doses result in marked bacteraemia and during the pre-mortal period the numbers of bacteria per 1 ml of blood amount to 100 and more. Such individuals may thus be a serious source of infection for blood sucking ectoparasites – vectors of tularaemia or their dead bodies and excrements may contaminate the environment. It seems, however, that not all hares die following the infection. Some develop a chronic form of the disease and become a permanent source of *F. tularensis* for other animals in the natural nidus as well as for humans. Interestingly, higher prevalence of tularaemia was found in females ($P = 0.05$). This fact is hard to explain because females are not supposed to have a higher risk of getting infected by *F. tularensis* than males.

Brucellosis of hares has only sporadically been reported in the studied area with specific antibodies being at the minimum. As shown by our results, brucellosis was more prevalent in adult animals compared to subadult ones ($P = 0.05$), which is understandable regarding its chronic nature and spread during reproduction. Brucellosis has primarily been caused by *Brucella suis* biotype 2 in the European brown hare. As shown (Szulowski et al., 1999), the European brown hare is a reservoir of this pathogen. Other free-living animals such as the wild boar or domestic animals, the pig in particular, may become infected in endemic areas. This fact was mentioned by Dedek (1983) and Hubalek et al. (1993, 2002) studying brucellosis in Germany and in the Czech Republic, respectively. The above authors found specific antibodies against *B. suis* in the wild boar and it is their opinion that the source of infection in endemic areas is the European brown hare, in particular. *B. suis* biotype 2 is considered to be non-pathogenic to humans. There is, however, a report of the infection of a farmer by this biotype (Teyssou et al., 1989), so the careful handling of animals is necessary.

Antibodies against leptospires were found in 7.5% of European brown hares in the studied area. It is thus clear that the European brown hare comes into contact with leptospires in its environment. Similar results were obtained by Sebek and Vosta (1958), Vosta (1961) and Asmera (1960) studying these matters in regions around Tabor, Jihlava and Ostrava, respectively. Treml and Nesnalova (1993) and Zitek and Babicka (2000) studied the occurrence of leptospirosis in wild animals, and like in our present survey they found the predomination of antibodies against *L. grippotyphosa*. Asmera (1991) confirmed this serotype in the European brown hare by culture. Similar findings were published by some authors from abroad (Hartman and Broekhuizen, 1980; Giraudo et al., 1985; Borcic et al., 1989; Dedek et al., 1990; Zanni et al., 1995). Serotype variability of leptospires found abroad in the European brown hare is, however, somewhat different from the Czech Republic and our results. It is due to the geographic differences in the occurrence of natural nidi of individual serotypes. Natural nidi of the *L. grippotyphosa* serotype are known to prevail under conditions of the Czech Republic; this fact being also documented in our study. This serotype is responsible for up to 90% of positive reactions in animals and humans (Sebek and Rosicky, 1974). The main reservoir of leptospires of this serotype is the common vole *(Microtus arvalis*), in which the seropositive cases amount up to 42.5% (Sebek and Vosta, 1958). Because this small rodent is widely distributed throughout biocoenoses in this country, natural nidi of leptospirosis of the *grippotyphosa* serotype can be expected anywhere in the Czech Republic and the European brown hare can get infected as confirmed in our study. The European

brown hare, however, is only an occasional reservoir of limited importance in maintaining the natural nidus of this serotype. It is nevertheless necessary to be careful in handling game animals potentially harbouring the infection, in particular when emptying the urinary bladder to prevent a direct contact of urine and skin injuries and abrasions because it is in urine that the leptospires are passed to the external environment.

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