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Authors: Hubálek, Z., Juticová, Z., Svobodová, Š., and Halouzka, J.

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A Serologic Survey for Some Bacterial and Viral Zoonoses in Game Animals in the Czech Republic

Z. Hubálek, Z. Juřicová, Š. Svobodová, and J. Halouzka, Academy of Sciences, Institute of Systematic and Ecological Biology, Květná 8, 60365 Brno, Czech Republic

ABSTRACT: Between 1986 and 1991, sera were collected from 33 roe deer (*Capreolus capreolus*), 24 red deer (*Cervus elaphus*), four fallow deer (*Dama dama*), two mouflon (*Ovis musimon*), 34 wild boars (*Sus scrofa*), and 48 hares (*Lepus europaeus*) shot in two areas of the Czech Republic. Collectively, the sera contained antibodies to *Coxiella burnetii* (prevalence of 12%), *Francisella tularensis* (4%), *Brucella* spp. (2%), Central European tick-borne encephalitis virus (8%), Ťahyňa (California serogroup) virus (36%), and Čalovo (=Batai) virus (23%). We propose that these mammals may play a role in maintaining natural foci of Q-fever, Ťahyňa fever and Čalovo virus infection.

Key words: Deer, mouflon, wild boar, hare, arbovirus infections, brucellosis, coxiellosis, tularemia, serologic survey.

Steadily increasing populations of roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), and wild boar (*Sus scrofa*) in the Czech Republic over the last 30 yr have caused concern to both forestry personnel and epizootiology officers. However, except for tularemia and brucellosis in the hares of some regions, regular serologic monitoring of game for zoonoses has been lacking in this country. Our objective was to determine the antibody prevalence of three bacterial and three viral zoonotic diseases in wild, hunter-killed mammals in the Czech Republic.

Blood samples were taken from 33 roe deer, 24 red deer, four fallow dear (*Dama dama*), two mouflon (*Ovis musimon*), 34 wild boars, and 48 hares (*Lepus europaeus*) in two regions. Eighteen red deer and 10 wild boars were shot in a hilly area in northern Bohemia (50°30′N, 13°30′ to 16°00′E) during 1986 and 1987; all other mammals were shot in the predominantly lowland countryside of southern Moravia (48°40′ to 49°30′N, 15°50′ to 17°00′E) during 1990 and 1991. The hares all were shot in December, while the other mammals were taken between May and November. The proportion of juveniles was very low in all species. The mean age for each species was as follows: roe deer, 4.4 (1 to 9) yr; red deer, 5.8 (1 to 15) yr; fallow deer, 4 (3 to 5) yr; mouflon, 5 (4 to 6) yr; and wild boar, 2.4 (1 to 7) yr. Sera were stored at −20 C until tested.

Coxiella burnetii antibodies were detected with the microagglutination test (MAT) (Fiset et al., 1969) using 25 μl of sera and phase II corpuscular hematoxylin-stained antigen (Bodibion MAR, Bioveta, Nitra, Slovak Republic). All sera were heated for 30 min at 56 C. A titer ≥1:8 was considered positive.

Francisella tularensis antibodies also were detected by MAT, using commercial antigen (Bioveta, Ivanovice, Czech Republic) stained with 0.005% safranin (Brown et al., 1980). Sera were not heated, and a titer ≥1:4 with typical agglutination reaction was considered positive. *Brucella* spp. antibodies were detected by standard tube agglutination test using 0.25 ml of sera and a commercial antigen of *B. abortus* (Bioveta, Ivanovice) according to the manufacturer's instructions.

Central European tick-borne encephalitis flavivirus (CEE) antigen was purchased (Imuna, Šarišské Michalany, Slovak Republic). Other arboviral antigens were prepared in this laboratory by saccharose-acetone extraction of infectious suckling mouse brains (Clarke and Casals, 1958): Ťahyňa virus (TAH) (California group, *Bunyaviridae*) strain 92, and Čalovo (=Batai) virus (CVO) (Bunyamwera group, *Bunyaviridae*) strain 184 were used. All sera were acetone-extracted, absorbed with goose erythrocytes and tested in the microhemagglutination inhibition test (HIT) (Clarke and Casals, 1958) with goose red

blood cells and four hemagglutinating units; a titer ≥1:20 was considered positive. All hare sera were examined additionally for TAH antibodies by a modified (Hubálek et al., 1979) plaque reduction neutralization test (PRNT) using Vero cells; we used about 50 plaque-forming units of the strain P6b of TAH virus per well, and considered an 80% plaque reduction as the titer value.

Antibodies to mosquito-borne agents (TAH, CVO) generally were more frequent (Table 1) than those against tick-borne agents (CEE, *Francisella*). The differences in antibody prevalence between CVO and CEE were significant ($P < 0.01$) with a chi-square test (Snedecor and Cochran, 1967). Antibodies to TAH virus were most common in roe deer, hares, and wild boar. These same mammalian species frequently had antibodies neutralizing TAH virus in neighboring Austria (Aspöck and Kunz, 1971). Danielová et al. (1969) found that 55% of the hares tested had antibodies neutralizing TAH virus in southern Moravia. In this study, all hare sera that reacted with TAH antigen in the HIT also were positive for TAH antibodies when tested in the PRNT; the titers in the PRNT ranged from 1:64 to 1:256). Eleven additional hares with antibodies (titers ranged from 1:32 to 1:64) were identified by the PRNT. Total TAH antibody prevalence of hares was therefore 67% in the PRNT as opposed to 44% in the HIT. Young hares are the principal amplifying host of TAH virus in natural foci of Central Europe; they develop viremias of sufficient level and duration to infect vector mosquitoes. The role of deer and wild boar is uncertain (Bárdoš, 1975; Rosický and Málková, 1980). We observed that 12 of 31 red deer and wild boar from the south Moravian lowlands (where mosquito vectors are abundant) had antibodies against TAH, but only two of 23 red deer and boar from the north Bohemian uplands (where mosquito vectors are infrequent) had TAH antibodies. Based on a chi-square test, this difference was significant ($P < 0.05$). Differences in

TABLE 1. Antibodies to six zoonotic agents in hunter-killed game animals in the Czech Republic, 1986–91.

Species	Antigen						
	Coxiella burnetii	*Francisella tularensis*	*Brucella abortus*	Central European tick-borne encephalitis virus	Tahyňa virus	Čalovo virus
Roe deer	2/33, (6), 32–64	2/33, (6), 4	0/33, (0), —	7/33, (21), 20–40	15/33, (45), 20–80	8/33, (24), 20–40
Red deer	6/24, (25), 8–128	1/24, (4), 4	0/24, (0), —	2/22, (9), 20–40	2/22, (9), 20–80	2/7, (29), 20–160
Fallow deer	2/4, (50), 16–128	0/4, (0), —	0/4, (0), —	0/4, (0), —	1/4, (25), 20	1/4, (25), 20
Mouflon	2/2, (100), 32–128	0/2, (0), —	0/2, (0), —	0/2, (0), —	0/2, (0), —	1/2, (50), 20
Wild boar	2/32, (6), 16	2/32, (6), 4	2/32, (6), 20–40	2/34, (6), 40	12/32, (38), 40–160	8/24, (33), 20–80
Hare	0/23, (0), —	0/23, (0), —	0/23, (0), —	1/48, (2), 20	21/48, (44), 40–160	5/38, (13), 20–160
Total	14/118, (11.9)	5/118, (4.2)	2/118, (1.7)	12/143, (8.4)	51/141, (36.2)	25/108, (23.1)

[a] Number seropositive/number tested, (% positive), range of reciprocal titers.

antibody prevalences between both regions with other antigens were insignificant, and may have been affected by the low numbers and proportions of reactors.

Antibodies to CVO virus often were detected in wild boars and deer, as Aspöck and Kunz (1971) found in Austria; in contrast, CEE antibodies were found more frequently in deer than in wild boars and hares. Deer of lowland populations are common hosts of adult and nymphal *Ixodes ricinus* ticks (Z. Hubálek and Š. Svobodová, unpubl. obs.), the principal vector of CEE virus.

A high proportion (29%) of red and fallow deer had Q-fever antibodies in titers up to 1:128; both mouflon examined also were positive. Based on this occurrence, we believe these species could pose a risk of infection to other wild animals, livestock, or even humans. *Coxiella burnetii* has been recovered from placental tissues of deer (*Odocoileus hemionus*) in California (USA) (Enright et al., 1971). Sheep living in the same area as the seropositive deer were tested with the MAT; 8 of 54 sheep tested had Q-fever antibodies, with titers ranging from 1:8 to 1:32).

Brucella agglutinating antibodies were demonstrated in two wild boars. It is possible that *B. suis* could have been responsible for this reaction, but cattle brucellosis caused by *B. abortus* has not occurred in this country for years; we did not confirm these results by other tests. A cross reaction with tularemia was excluded; no sera reacting with *Brucella* agglutinated *Francisella tularensis* antigen. However, *B. abortus* antigen can cross-agglutinate partially with the antibodies against such bacteria as *Yersinia enterocolitica* serotype 0:9 and *Proteus* OX19 (Mittal and Tizard, 1981; Corbel and Brinley-Morgan, 1984). Antibodies against *Francisella* were rare and of low titer (1:4) in the mammals, though *F. tularensis* was isolated from ixodid ticks in the study area (Hubálek et al., 1990).

We propose that some of these mammals may play a role in maintaining enzootic foci of Q-fever, Ťahyňa (California encephalitis) and Čalovo virus infection in the Czech Republic. These wildlife also may be good indicator species for evaluating human risk to these zoonotic agents.

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