Serological survey of the wild boar (*Sus scrofa*) for tularaemia and brucellosis in South Moravia, Czech Republic

Z. Hubálek[1], F. Treml[2], Z. Juřicová[1], M. Huňady[3], J. Halouzka[1], V. Janík[4], D. Bill[4]

[1]Medical Zoology Laboratory, Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

[2]Institute of Infectious Diseases and Epizootiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

[3]Bioveta a.s., Ivanovice na Hané, Czech Republic

[4]District Veterinary Service, Břeclav, Czech Republic

ABSTRACT: Sera of 204 wild boars (*Sus scrofa*), shot by hunters in the South-Moravian district of Břeclav during 1993-2001, were tested by microagglutination reaction using safranin-stained antigens of *Francisella tularensis* and *Brucella abortus*: 10.8% and 8.7% seroreactors, respectively, were detected. The highest (17%) prevalence of tularaemia antibodies was found in wild boars during 1993–1994 at the beginning of a widespread outbreak of tularaemia in South Moravia that started in 1994, a nonsignificantly lower (13%) seroprevalence in 1995–1996 during the continuing epizootic, whereas it decreased markedly to 3% in the years 1997–2001 during the disappearance of the epizootic. *Brucella* sp. antibodies were significantly most frequent (15%) in wild boars in the years 1995–1996. This *Brucella* seroreactivity has been attributed to *B. suis* biotype 2 (*B. melitensis* biovar Suis biotype 2 according to new nomenclature) infection, because *B. abortus* in both cattle and humans (Bang's disease) was eradicated in the former Czechoslovakia in 1964. The hare brucellosis (*B. suis* biotype 2) has occurred in the Břeclav district in a number of natural foci revealing an increased activity since 1994.

Keywords: *Francisella tularensis*; *Brucella melitensis* biovar Suis; *Brucella suis*; *Sus scrofa*; wild boar; wild swine; wildlife; game animals; serosurvey; microagglutination test; complement fixation test; zoonoses; natural foci of diseases

Natural foci of tularaemia and brucellosis (the etiologic agents are *Francisella tularensis* and *Brucella suis* biotype 2, respectively) have been detected during the autumn hunting of hares (*Lepus europaeus*) in the Břeclav district of South Moravia (Czech Republic) at different extent and intensity virtually every year since the 1960s (data of District Veterinary Service). However, while all shot hares in the district are examined routinely for these two zoonoses, the other species of game animals escape this screening. A limited testing of deer, mouflon and wild boar was done in 1990, and the seroprevalence for both tularaemia and brucellosis was found to be up to 6% of the animals (Hubálek *et al.*, 1993).

An increased activity of natural foci of tularaemia was reported in southern Moravia and adjacent regions of Slovakia and Austria in autumn 1994 (Hubálek *et al.*, 1996, 1997; Treml *et al.*, 1997, 2001; Guryčová *et al.*, 1999, 2001). After the isolation of *F. tularensis* from ixodid ticks and rodents, we decided to carry out a serosurvey of locally abundant wild boar (*Sus scrofa*), a potential host or reservoir of many zoonoses (Fenske and Pulst, 1973; Becker *et al.*, 1978; Zygmont *et al.*, 1982; Dedek *et al.*, 1986; Robson *et al.*, 1993; Edelhofer *et al.*, 1996; Gibbs, 1997; Saliki *et al.*, 1998; Deutz and Köfer, 1999; Heinritzi *et al.*, 1999). In parallel, we tested the sera for antibodies against *Brucella* because

Supported by the Grant Agency of the Czech Republic (Grant No. 524/00/1122) and the Grant Agency Academy of Sciences of the Czech Republic (Grant No. S6093007).

of the agglutination cross-reactivity between the two microbial genera.

MATERIAL AND METHODS

Blood samples collection

Wild boars were killed by hunters in a number of localities in the district of Břeclav from 1993 to 2001. During the veterinary inspection of the shot animals, the blood samples were collected from the heart or thoracic cavity into plastic tubes and allowed to clot. The sera were then separated by centrifugation and stored at –20°C until tested. Strongly haemolytic sera were discarded.

Microagglutination test (MAT)

Examination of sera for both tularaemia and brucellosis was carried out in plastic microplates with U-shaped wells, using slow agglutination reaction and commercial antigens of *Francisella tularensis* and *Brucella abortus* (Bioveta a.s., Ivanovice na Hané, Czech Republic) that were stained in our laboratory by safranin O at a final concentration of 0.005% (Brown *et al.*, 1980). In each well, 25 µl of the stained antigen (*F. tularensis* or *B. abortus*), diluted five times with saline, was mixed with 25 µl of sera diluted serially two-fold with saline starting from 1 : 5. The controls included commercial immune sera against tularaemia and brucellosis (Bioveta): tularaemia serum titered against antigens of *F. tularensis* and *B. abortus* at 1 : 40 and <1 : 5, respectively, while brucella serum reacted against the same antigens at 1: 10 and 1: 320, respectively. The microplates were gently shaken, placed in an incubator at 37°C for 4 hours, and then at +4°C overnight for a final reading. Sera positive (with a typical agglutinate in a dilution of at least 1 : 10) in MAT were checked in a standard agglutination test (Francis and Evans, 1926) on WHO plates or in glass tubes, using 200-µl volumes of serum and unstained antigen *F. tularensis* or *B. abortus* (Bioveta).

Complement-fixation test (CFT)

Sera reacting with *B. abortus* in MAT were additionally examined with CFT in tubes according to the manufacturer's (Bioveta) instructions. The sera were first inactivated at 60°C for 30 min; however, a number of them coagulated and were unsuitable for the CFT procedure.

Statistical evaluation

Differences in antibody prevalence between various groups of animals were evaluated with the chi-square and Fisher exact tests, and coincidence in long-term prevalence between tularaemia and brucellosis in hares was tested with Pearson, Spearman and Kendall coefficients of correlation (Snedecor and Cochran, 1967). The differences and correlation values with the probability of the null hypothesis $P < 0.05$ were regarded as significant.

RESULTS

Of 204 wild boars examined in MAT, 22 animals (10.8%) reacted with *F. tularensis* (the titres varied between 1 : 10 to 1 : 40) while 18 animals (8.7%) with *B. abortus* (titres from 1 : 10 to 1 : 80; Table 1). Cross-reactions between tularaemia and brucellosis occurred

Table 1. A survey of examined wild boars according to year and age groups

	Number of examined wild boars	*F. tularensis* MAT positive	*B. abortus* MAT positive
Total	204	22 (10.8%)	18 (8.7%)
Period: 1993–1994	36	6 (16.7%)	0 (0.0%)
1995–1996	108	14 (13.0%)	16 (14.8%)
1997–2001	60	2 (3.3%)	2 (3.3%)
Age: <1year	57	6 (10.5%)	4 (7.0%)
1 year	78	10 (12.8%)	9 (11.5%)
>1 year	36	6 (16.7%)	3 (8.3%)

only exceptionally and it was possible to identify the corresponding agent according to the titre levels. It cannot be excluded, however, that dual infection sometimes occurred.

The localities and numbers of animals with antibodies to *F. tularensis*/*B. abortus* were: Boleradice 1/0, Břeclav 1/0, Bulhary 4/2, Charvatská Nová Ves 3/2, Hlohovec 1/1, Hustopeče 0/1, Lanžhot 3/0, Lednice 1/1, Moravská Nová Ves 2/2, Poštorná 2/3, Rakvice 1/1, Valtice 2/4, Velký Dvůr 0/1, Vranovice 1/0.

Analysis of the results according to year (Table 1) has shown the highest prevalence of antibodies to tularaemia (17% of animals) in 1993–1994, i.e. at the beginning of the tularaemia outbreak in southern Moravia, a nonsignificantly lower one (13%) in 1995–1996 during the continuing epizootic, whereas a significantly decreased prevalence (3%) in the years 1997 through 2001 during a reduced activity of tularaemia in the region. A similar analysis for brucellosis has detected the highest seroprevalence (15%) in the years 1995–1996.

A moderate but statistically insignificant increase of tularaemia antibody prevalence was observed with growing age of wild boars, whereas the highest proportion of animals with antibodies to brucellosis was interestingly found among yearlings (Table 1).

Only five sera reacting with *Brucella* antigen in MAT could have also been examined with *B. abortus* antigen in CFT (Table 2) – the reasons have been described in Material and Methods. Four of the animals were confirmed as having antibodies to *Brucella*, whereas the boar no. 475 had antibodies reacting in MAT with both *F. tularensis* and *B. abortus*, and it had probably been infected with *F. tularensis*. These data indicate that MAT with the *B. abortus* antigen gives specific results as far as a serum does not react simultaneously with *F. tularensis*.

Table 2. Reciprocal titres of antibodies to *B. abortus* and *F. tularensis* in microagglutination (MAT) and complement fixation (CFT) tests in five wild boars

Animal No.	*B. abortus* MAT	*B. abortus* CFT	*F. tularensis* MAT
454	80	160	<10
475	40	<10	40
493	80	80	<10
498	20–40	20	<10
499	10–20	80	<10

DISCUSSION

Sensitivity and specificity of MAT with stained antigen have been demonstrated to be comparable or superior to those of the standard tube agglutination test with unstained antigen (Francis and Evans, 1926) in both *Francisella* (Massey and Mangiafico, 1974; Brown *et al.*, 1980; Sato *et al.*, 1990) and *Brucella* (Bettelheim *et al.*, 1983; Moyer *et al.*, 1987; Rogers *et al.*, 1989; Sato *et al.*, 1990). We can confirm that MAT compared with the standard tube agglutination 'macrotest' is quicker, easier to perform, more economical (saving sera and antigens) as well as better readable when the sera are haemolytic.

Dedek *et al.* (1986) examined 1061 wild boars in Germany for antibodies against tularaemia (0.1% were positive) and brucellosis (7.9% positive). A much higher seroprevalence to *Brucella* has recently been found in France: overall, 31.6% of 2313 wild boars were positive between 1994 and 2000 (Garin-Bastuji and Delcueillerie, 2001). The detection of antibodies to *B. abortus* in wild boars should be interpreted with care, taking into account cross-reactivity among *Brucella*, *Francisella* and *Yersinia enterocolitica* 0 : 9 (Mittal and Tizard, 1980). We did not test the sera against the latter microorganism due to inaccessibility of the antigen. Moreover, in respect to the well-known complete cross-agglutination reactivity of *Brucella* spp. (according to DNA hybridization the genus *Brucella* is, in fact, monospecific with the only species named *B. melitensis* involving five biovars – Abortus, Canis, Neotomae, Ovis, Suis: Verger *et al.*, 1985) it is most probable that our results do not indicate the presence of *B. abortus*, the etiologic agent of Bang's disease which was eradicated in the former Czechoslovakia in 1964, but that they demonstrate infection of wild boars with *B. suis* biotype 2 (according to new nomenclature *B. melitensis* biovar Suis, biotype 2), i.e. the agent of hare brucellosis which occurs in the Břeclav district (Štěrba, 1982; data of District Veterinary Service, Břeclav). *Brucella abortus* has not been isolated from either domestic or wild animals in the Czech Republic since the 1970s (data of the State Veterinary Service, Prague).

Brucella suis biotype 2 is distributed in many other European countries: Slovakia (Nižnánsky *et al.*, 1957), Hungary (Kormendy and Nagy, 1982), Austria (Willinger, 1960; Damoser and Hofer, 1995; Höflechner-Pöltl *et al.*, 2000), Germany (Dedek, 1983; Kautzsch *et al.*, 1995; Heinritzi *et al.*, 1999), Switzerland (Haerer *et al.*, 2001), France (Teyssou *et al.*, 1989; Garin-Bastuji and Delcueillerie, 2001), Belgium

(Francart *et al.*, 1983; Godfroid *et al.*, 1994), Denmark (Thomsen, 1957, 1959; Thimm, 1982), Poland (Szulowski, 1999), Slovenia (Brglez and Batis, 1981), Yugoslavia (Thimm, 1982) and, after the introduction of European hares, even in Argentina (Szyfres *et al.* 1968). Two strains of *B. suis* biotype 2 were isolated from wild boars near Potsdam in Germany (Fenske and Pulst, 1973), and additional strains were recovered from this animal species in Bavaria (Heinritzi *et al.*, 1999), Austrian Styria (Deutz and Köfer, 1999), Belgium (Godfroid *et al.*, 1994) and France (34 isolations between 1994 and 2000: Garin-Bastuji and Delcueillerie, 2001). On the other hand, *B. suis* biotype 1 predominates in feral swine in other parts of the world (Becker *et al.*, 1978; Zygmont *et al.*, 1982; Corn *et al.*, 1986; Robson *et al.*, 1993; Gibbs, 1997).

The increased prevalence of *Brucella* antibodies in wild boars in the years 1995-1996 followed a markedly growing activity of hare brucellosis in the Břeclav district since 1994 (Table 3). Interestingly, brucellosis was diagnosed in domestic pigs monitored in the Czech Republic between 1992 and 2000 only in 1994 when 70 (0.05%) of 154 319 animals seroreacted, and 4 of 107 seropositive pigs also yielded *Brucella* sp. by cultivation (Kolbabová *et al.*, 2001). In general, there was a considerable parallel in the incidence between brucellosis and tularemia in local hares from 1990 to 2000 (Table 3), with high correlation coefficient values ($P < 0.001$) of Pearson $r = 0.934$, Spearman $\varrho = 0.934$ and Kendall $\tau = 0.807$. Significant correlations were also found between the disease incidence in hares and the number of corresponding foci (Table 3) for both tularaemia ($r = 0.705$; $\varrho = 0.650$; $\tau = 0.509$) and brucellosis ($r = 0.628$; $\varrho = 0.700$; $\tau = 0.491$). Pikula (1996) found that the numbers of natural foci in areas endemic for tularaemia correlate with the population level of the European hare. We can therefore suppose that the growing incidence of both tularaemia and hare brucellosis were associated with the increasing population density of hares (as revealed by the numbers of animals shot in the district between 1990 and 2000).

The wild boar is an omnivorous species that feeds even on carrion; some individuals could thus come into contact with infected dead hares or their aborted foetuses (Damoser and Hofer, 1995). Along with the hare, the wild boar is regarded as the natural reservoir of *B. suis* biotype 2 in Europe (Dedek, 1983; Wilhelm and Zeiris, 1985; Kautzsch *et al.*, 1995; Szulowski, 1999; Szulowski and Pilaszek, 2000; Garin-Bastuji and Delcueillerie, 2001). This bacterium is pathogenic to the hare (Vítovec *et al.*, 1976; Štěrba, 1982), wild boar and domestic pig (Nicolet *et al.*, 1979; Köhler and Wille, 1980; Godfroid *et al.*, 1994; Kautzsch *et al.*, 1995; Heinritzi *et al.*, 1999; Garin-Bastuji and Delcueillerie, 2001).

Brucella suis, including biotype 2, has been reported with an increasing frequency as the causative agent of human disease (Chastel *et al.*, 1970; Joubert *et al.*, 1970; Golden *et al.*, 1970; Heineman and Dziamski, 1976; Morris *et al.*, 1979; Nadler *et al.*, 1982; Thimm, 1982; Williams and Crossley, 1982; Francart *et al.*, 1983; Teyssou *et al.*, 1989; Bergeron *et al.*, 1992; Robson *et al.*, 1993; Kant *et al.*, 1994; Paton *et al.*, 2001; Kolbabová *et al.*, 2001). We previously found *Brucella* antibodies in 5.2% of 524 adult women attending outpatient clinics and hospital in the Břeclav district in 1985–1986:

Table 3. Incidence of tularaemia and brucellosis in the hunter-killed hare (*Lepus europaeus*): Břeclav district, 1990–2000. (Data from the District Veterinary Service Břeclav). [The figures in brackets show the number of foci in the district]

Year	Number of hares killed and examined	Positive for	
		tularaemia	brucellosis
1990	7 398	0.61% [47]	0.01% [7]
1991	5 089	0.96% [52]	0.04% [1]
1992	7 264	0.77% [59]	0.12% [4]
1993	7 875	0.61% [49]	0.00% [8]
1994	14 024	5.75% [62]	0.72% [18]
1995	5 307	3.75% [57]	0.47% [32]
1996	5 402	2.28% [57]	0.35% [38]
1997	6 584	4.53% [59]	0.76% [39]
1998	6 829	3.44% [60]	0.31% [34]
1999	8 591	2.11% [61]	0.09% [29]
2000	11 842	3.36% [62]	0.30% [27]

one of these aborted, and another gave a premature birth to a baby with congenital malformation (microcephaly); because all other results of examination in these two women were negative, the symptoms might have been related to infection with *B. suis* biotype 2 (Hubálek *et al.*, 1987).

Infection of wild boars with tularaemia (probably asymptomatic, but piglets are reported to be quite susceptible to the disease), might occur either by the oral route or via the vector, *Dermacentor reticulatus*. This tick species is distributed in certain habitats along the lower reaches of the rivers Dyje and Morava of the Břeclav district, their adult stages parasitize wild boars commonly, and the rate of tick infection with *F. tularensis* can be quite high during epizootics, 1% to 4% (Hubálek *et al.*, 1996).

Acknowledgement

We are indebted to MVDr. J. Hnilička, the former Director of the District Veterinary Service Břeclav for the support, and to a number of hunters and veterinarians (MVDr. P. Forejtek, MVDr. J. Minář, MVDr. K. Peštál, staff members of the District Veterinary Service Břeclav) for the collection of blood specimens. Sera of 17 wild boars shot in the Břeclav district were kindly supplied by the State Veterinary Institute in Jihlava (the former Director MVDr. J. Holejšovský, MVDr. P. Barták and MVDr. I. Kucharovičová). MVDr. Š. Svobodová was involved in the early serological investigations of the animals.

REFERENCES

Becker H.N., Belden R.C., Breault T., Burridge M.J., Frankenberger W.B., Nicoletti P. (1978): Brucellosis in feral swine in Florida. J. Am. Vet. Med. Assoc., *173*, 1181–1182.

Bergeron P., Gonzales-Fajardo J., Mangialardi N., Courbier R. (1992): False aneurysm of the abdominal aorta due to *Brucella suis*. Ann. Vasc. Surg., *6*, 460–463.

Bettelheim K.A., Maskill W.J., Pearce J. (1983): Comparison of standard tube and microagglutination techniques for determining *Brucella* antibodies. J. Hyg., *90*, 33–39.

Brglez I., Batis J. (1981): Brucellosis in wild hares in Slovenia. Vet. Glas., *6*, 555–560.

Brown S.L., McKinney F.T., Klein G.C., Jones W.L. (1980): Evaluation of a safranin-O-stained antigen microagglutination test for *Francisella tularensis* antibodies. J. Clin. Microbiol., *11*, 146–148.

Chastel C., Roux J., Etienne J., Bereni J. (1970): First case of human brucellosis due to *Brucella suis* observed in France. Sem. Hop., *46*, 254–256.

Corn J.L., Swiderek P.K., Blackburn B.O., Erickson G.A., Thiermann A.B., Nettles V.F. (1986): Survey of selected diseases in wild swine in Texas. J. Am. Vet. Med. Assoc., *189*, 1029–1032.

Damoser J., Hofer E. (1995): *Brucella suis* Biovar 2-Infektionen beim Feldhasen. Z. Jagdwiss., *41*, 137-141.

Dedek J. (1983): Zur Epizootiologie der Schweinebrucellose unter besonderer Berücksichtigung von Erregerreservoiren. Mh. Vet. –Med., *39*, 845–849.

Dedek J., Löpelmann H., Natterman A. (1986): Serologische Untersuchungen auf Brucellose und Tularämie beim Schwarzwild. Mh. Vet. -Med., *41*, 150–153.

Deutz A., Köfer J. (1999): Schwein und Wildschwein als Träger von Zoonosen. Berl. Münch. Tierärztl. Wschr., *112*, 305–310.

Edelhofer R., Prosl H., Kutzer E. (1996): Trichinellose und Toxoplasmose in Wildschwein aus Ost-Österreich. Wien. Tierärztl. Mschr., *83*, 225–229.

Fenske G., Pulst H. (1973): Die epizootiologische Bedeutung der Hasen- und Wildschwein-Brucellose. Mh. Vet.-Med., 28, 537–541.

Francart H., Segaert M., Verlinde A., Castryck F., Dekeyser J., Vandepitte J. (1983): Human infection with *Brucella suis* biotype in Belgium. Méd. Mal. Infect., *13*, 287–289.

Francis E., Evans A.C. (1926): Agglutination, cross-ag-glutination, and agglutinin absorption in tularaemia. Publ. Hlth. Rep., *41*, 1273–1295.

Garin-Bastuji B., Delcueillerie F. (2001): Les brucelloses humaine et animale en France en l'an 2000. Méd. Mal. Infect., *31*, Suppl. 2, 202–216.

Gibbs E.P.J. (1997): The public health risk associated with wild and feral swine. Rev. Sci. Tech. Off. Int. Epizoot., *16*, 594–598.

Godfroid J., Michel P., Uytterhaegen L., Desmedt C., Rasseneur F., Boelaert F., Saegerman C., Patigny X. (1994): Brucellose enzootique à *Brucella suis* biotype 2 chez le sanglier (*Sus scrofa*) en Belgique. Ann. Méd. Vet., *138*, 263–268.

Golden B., Layman T.E., Koontz F.P., Mergner W.J. (1970): *Brucella suis* endocarditis. South Med. J., *63*, 392–395.

Guryčová D., Varga V., Výrosteková V., Gacíková E., Péči J. (1999): An epidemic of tularaemia in West Slovakia in 1995–1996 (in Slovak). Epidemiol. Mikrobiol. Imunol., *48*, 97–101.

Guryčová D., Výrosteková V., Khanakah G., Kocianová E., Stanek G. (2001): Importance of surveillance of tularemia natural foci in the known endemic area of Central Europe, 1991–1997. Wien. Klin. Wschr., *113*, 433–438.

Haerer G., Nicolet J., Bacciarini L., Gottstein B., Giacometti M. (2001): Todesursachen, Zoonosen und Reproduktion

bei Feldhasen in der Schweiz. Schweiz. Arch. Tierheilk., *143*, 193–201.

Heineman H.S., Dziamski I.M. (1976): *Brucella suis* infection in Philadelphia. A survey of hog fever and asymptomatic brucellosis. Am. J. Epidemiol., *103*, 88–100.

Heinritzi K., Aigner K., Erber M., Kersjes C., von Wangenheim B. (1999): Brucellosis and Aujeszky's disease (pseudorabies) in wild boars within an enclosure (case report). Prax. Ausg. Grobtiere Nutztiere, *27*, 50–55.

Höflechner-Pöltl A., Hofer E., Awad-Masalmeh M., Müller M., Steineck T. (2000): Tularämie und Brucellose bei Feldhasen und Füchsen in Österreich. Tierärztl. Umsch., *55*, 264–268.

Hubálek Z., Chalupský V., Juřicová Z., Ševčíková L. (1987): Serological examination of women for tularaemia and brucellosis in the district of Břeclav (in Czech). Čas. Lék. Čes., 1*26*, 871–873.

Hubálek Z., Juřicová Z., Svobodová Š., Halouzka J. (1993): A serologic survey for some bacterial and viral zoonoses in game animals in the Czech Republic. J. Wildl. Dis., *29*, 604–607.

Hubálek Z., Treml F., Halouzka J., Juřicová Z., Huňady M., Janík V. (1996): Frequent isolation of *Francisella tularensis* from *Dermacentor reticulatus* ticks in an enzootic focus of tularaemia. Med. Vet. Entomol., *10*, 241–246.

Hubálek Z., Sixl W., Halouzka J., Mikulášková M. (1997): Prevalence of *Francisella tularensis* in *Dermacentor reticulatus* ticks collected in adjacent areas of the Czech and Austrian Republics. Centr. Eur. J. Publ. Hlth., *5*, 199–201.

Joubert L., Rancien P., Sendral R., Prave M. (1970): Un cas de brucellose du lievre à *Brucella suis* dans la Saone-et-Loire. Bull. Soc. Sci. Vet. Med. Comp. Lyon, *72*, 186–187.

Kant G.D., van Lijf J.H., Karthaus R.P. (1994): Spondylodiscitis caused by *Brucella suis*. Ned. Tijdschr. Geneeskd., *138*, 2584–2587.

Kautzsch S., Seyfarth D., Schöne R., Stehmann R. (1995): Ein Fall von Brucellose der Schweine und daraus abgeleitete Überlegungen zur Epidemiologie dieser Tierseuche. Berl. Münch. Tierärztl. Wschr., *108*, 201–205.

Köhler B., Wille H. (1980): Bakteriologische Untersuchungen bei abortierten Schweinfeten unter besonderer Berücksichtigung der ätiologischen Bedeutung von *Staphylococcus aureus*. Mh. Vet.-Med., *35*, 506–510.

Kolbabová J., Havlasová J., Veleba J. (2001): Brucellosis (in Czech). Klin. Mikrobiol. Inf. Lék., *7*, 210–213.

Kormendy B., Nagy G. (1982): The supposed involvement of dogs carrying *Brucella suis* in the spread of swine brucellosis. Acta Vet. Acad. Sci. Hung., *30*, 3–7.

Massey E.D., Mangiafico J.A. (1974): Microagglutination test for detecting and measuring serum agglutinins of *Francisella tularensis*. Appl. Microbiol., *27*, 25–27.

Mittal K.R., Tizard I.R. (1980): Studies on the relationships between *Yersinia enterocolitica* IX and *Brucella abortus* agglutinins in naturally infected animals. Res. Vet. Sci., *28*, 311–314.

Morris S.J., Greenwald R.A., Turner R.L., Tedesco F.J. (1979): *Brucella*-induced cholecystitis. Am. J. Gastroenterol., *71*, 481–484.

Moyer N.P., Evins G.M., Pigott N.E., Hudson J.D., Farshy C.E., Feeley J.C., Hausler W.J. (1987): Comparison of serologic screening tests for brucellosis. J. Clin. Microbiol., *25*, 1969–1972.

Nadler H., Dolan C., Forgacs P., George H. (1982): *Brucella suis* – an unusual cause of suppurative lymphadenitis in an outpatient. J. Clin. Microbiol., *16*, 575–576.

Nicolet J., Schmid H.R., Studer H., Dauwalder M. (1979): Ein Ausbruch von *Brucella suis* Biotype 2 Infektion beim Schwein. Schweiz. Arch. Tierheilk., *121*, 231–238.

Nižnánsky F., Gmitter J., Ofúkaný L. (1957): Beitrag zur Epizootologie der Brucellose der Feldhasen in der Slowakei. In: Nižnánsky F. (ed.): Die Fortschritte in der Brucelloseforschung. SAV, Bratislava. 196–200.

Paton N.I., Tee N.W., Vu C.K., Teo T.P. (2001): Visceral abscesses due to *Brucella suis* infection in a retired pig farmer. Clin. Infect. Dis., *32*, 129–130.

Pikula J. (1996): Ecological and epizootiological studies of tularemia in the European Hare, *Lepus europaeus* (in Czech). [Ph. D. Thesis.] University of Veterinary and Pharmaceutical Sciences, Brno. 89 pp.

Robson J.M., Harrison M.W., Wood R.N., Tilse M.H., McKay A.B., Brodribb T.R. (1993): Brucellosis – reemergence and changing epidemiology in Queensland. Med. J. Aust., *159*, 153–158.

Rogers R.J., Cook D. R., Ketterer P.J., Baldock F.C., Blackall P.J., Stewart R.W. (1989): An evaluation of three serological tests for antibody to *Brucella suis* in pigs. Aust. Vet. J., *66*, 77–80.

Saliki J.T., Rodgers S.J., Eskew G. (1998): Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. J. Wildl. Dis., *34*, 834–838.

Sato T., Fujita H., Ohara Y., Homma M. (1990): Microagglutination test for early and specific serodiagnosis of tularemia. J. Clin. Microbiol., *28*, 2372–2374.

Snedecor G.W., Cochran W.G. (1967): Statistical Methods. 6th ed. The Iowa State University Press, Ames. 593 pp.

Štěrba F. (1982): Pathomorphological changes in brucellosis in hares (in Czech). Vet. Med. (Praha), *27*, 437–448.

Szulowski K. (1999): Diagnosis of *Brucella suis* infections in pigs and hares by ELISA. Pol. J. Vet. Sci., *2*, 65–70.

Szulowski K., Pilaszek J. (2000): Wild animals as reservoirs of *Brucella*. Med. Wet., *56*, 687–690.

Szyfres B., Gonzalez Tome J., Palacio Mendieta T. (1968):

Isolation of *Brucella suis* from the European hare (*Lepus europaeus*) in Argentina. Bol. Oficina Sanit. Panam., *65*, 441–445.

Teyssou R., Morvan J., Leleu J.P., Roumegou P., Goullin B., Carteron B. (1989): About a case of human brucellosis due to *Brucella suis* biovar 2. Méd. Mal. Infect., *19*, 160–161.

Thimm B.M. (1982): Brucellosis. Springer, Berlin-Hei-delberg-New York. 55 pp.

Thomsen A. (1957): Brucellainfektion beim Schwein und ihre Beziehung zur Brucellainfektion beim Hasen. In: Nižnánsky F. (ed.): Die Fortschritte in der Brucellose-forschung. SAV, Bratislava. 184–193.

Thomsen A. (1959): Occurrence of *Brucella* infection in swine and hares with special regard to the European countries. Nord. Vet.-Med., *11*, 709–718.

Treml F., Hubálek Z., Halouzka J., Juřicová Z., Huňady M., Janík V., Bill D. (1997): Activation of a natural focus of tularaemia (in Czech). Veterinářství, No. 2, 49–51.

Treml F., Hubálek Z., Halouzka J., Juřicová Z., Huňady M., Janík V. (2001): An analysis of incidence of tularaemia in the district of Břeclav, 1994–1999 (in Czech). Epidemiol. Mikrobiol. Imunol., *50*, 4–9.

Verger J.M., Grimont F., Grimont P.A. D., Grayon M. (1985): *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. Int. J. Syst. Bact., *35*, 292–295.

Vítovec J., Vladík P., Záhor P., Slabý V. (1976): Pathological studies on 70 cases of brucellosis in hares caused by *Brucella suis* (in Czech). Vet. Med. (Praha), *21*, 359–368.

Wilhelm A., Zieris H. (1985): Das Wildschwein (*Sus scrofa scrofa*) als primäres Naturreservoir für *Brucella suis*. Erkrank. Zootiere, *27*, 463–467.

Williams R.K., Crossley K. (1982): Acute and chronic hepatic involvement of brucellosis. Gastroenterology, *83*, 455–458.

Willinger H. (1960): Brucellose bei Feldhasen in Österreich. Wien. Tierärztl. Mschr., *47*, 661–669.

Zygmont S.M., Nettles V.F., Shotts E.B., Carmen W.A., Blackburn B.O. (1982): Brucellosis in wild swine: a serologic and bacteriologic survey in the southeastern United States and Hawaii. J. Am. Vet. Med. Assoc., *181*, 1285–1287.

Received: 02–01–16
Accepted after corrections: 02–03–01

Corresponding Author

Doc. RNDr. Zdeněk Hubálek, DrSc., Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Klášterní 2, 691 42 Valtice, Czech Republic
Tel. +420 6 27 35 29 61, fax +420 6 27 35 23 87, e-mail: zhubalek@brno.cas.cz