Detection of bovine and human tuberculosis in cattle and other animals in six Central European countries during the years 2000–2004

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ABSTRACT: During a five year period (2000–2004), the incidence of bovine and human tuberculosis in cattle and other animals from six Central European countries was evaluated: Croatia (HRV), the Czech Republic (CZE), Hungary (HUN), Poland (POL), Slovakia (SVK), and Slovenia (SVN). These countries, lying between the Baltic and the Adriatic seas, cover an area of 610 402 km^2 and had more than 68 million inhabitants and 9 330 264 cattle in 2003. Successful national control programmes against bovine tuberculosis in cattle were carried out up until 1980: HRV (1953–1966), CZE (1959–1968), HUN (1962–1980), POL (1959–1975) SVK (1959–1968), and SVN (1962–1973). During the entire monitored period skin testing with bovine tuberculin in all cattle older than two years was carried out regularly either once a year or every second year. Five of these countries (CZE, HUN, POL, SVK, and SVN) joined the EC on May 1st, 2004. CZE and SVK were officially declared free of bovine tuberculosis in cattle on March 31st, 2004 (Commission Decision No. 2004/320/EC) and March 4th, 2005 (Commission Decision No. 2005/179/EC), respectively. Bovine tuberculosis was diagnosed in a total of 188 cattle herds in the study area: 145 (77.1%) outbreaks in small herds (< 10 cows) and 43 (22.9%) outbreaks in large herds (≥ 10 cows). The last cases of bovine tuberculosis in cattle in SVK and in the CZE were diagnosed in 1993 and 1995, respectively. During the monitored period, bovine tuberculosis was diagnosed in animal species other than cattle found in zoological gardens: in two Bactrian camels (*Camelus ferus*) in the CZK, in one Siberian tiger (*Panthera tigris* f. *altaica*) in HUN, in one bison (*Bison bison*) and one eland (*Taurotragus oryx*) in POL, and in one dromedary camel (*Camelus dromedarius*) and two bison in SVN. In wild animals, bovine tuberculosis was diagnosed in wild boar (*Sus scrofa*) in HUN (n = 14) and HRV (n = 1), in six red deer (*Cervus elaphus*) in HUN, in 14 European bison (*Bison bonasus* f. *bonasus*) and two roe deer (*Capreolus capreolus* f. *capreolus*) in POL. Infections caused by *Mycobacterium tuberculosis* were also diagnosed in four cattle and two pigs in POL, in one cattle in SVN, in one dog (*Canis lupus* f. *familiaris*) in the CZE and in one dog in HUN. *M. africanum* was diagnosed in one hyrax (*Procaria habessinica*) in a zoological garden in HRV.

Keywords: veterinary epidemiology; zoonosis; *Mycobacterium caprae*; human tuberculosis; bovine tuberculosis

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Bovine tuberculosis caused by *Mycobacterium bovis* and *M. caprae* in Europe still remains one of the most serious diseases in both humans and animals. Due to the fact that tuberculosis is a notifiable disease, the yearly incidence of bovine tuberculosis in cattle is reported in statistical evaluations prepared by the Office International des Epizooties (OIE) for all European countries. Bovine tuberculosis outbreak situations differ in respective European countries. With respect to the International Animal Health Code OIE definition, a region is free of bovine tuberculosis in cattle provided prevalence of infected herds does not exceed 0.2% of all kept herds.

The European Commission Decision 97/76/EC of 17 December 1996 laid down the methods of control for maintaining the officially tuberculosis free status of bovine herds in certain Member States and regions of Member States (Anonymous, 1997). Member states and regions of member states are free of bovine tuberculosis if at least 99.9% of the cattle herds have been declared officially tuberculosis free for at least 10 years and where every year (for at least six years) bovine tuberculosis has not been found to be present in more than one herd per 10 000 herds in that Member State or part thereof.

Based on the Commission Decision 1999/467/EC of 15 July 1999 seven states of the European Union were classified as free of bovine tuberculosis: Denmark, Germany, Luxembourg, the Netherlands, Austria, Finland, and Sweden (Anonymous, 1999). In 2004 Belgium, the Czech Republic, and France were added to this group (Anonymous, 2004) and on 4th March 2005 an eleventh country, Slovakia, was added to these states (Anonymous, 2005).

National control programmes against bovine tuberculosis were successfully carried out between 1950 and 2000 in the majority of Central European countries (Table 1). Between 1990 and 1999, the reported incidence of bovine tuberculosis in cattle decreased in all six countries in the study area (Pavlik et al., 2002a): Croatia, the Czech Republic, Hungary, Poland, Slovakia, and Slovenia. The incidence of bovine tuberculosis was not only reduced in cattle, but in other animals that predominantly originated from zoological gardens (Pavlik et al., 1998, 2002e). Of the eight members of the *Mycobacterium tuberculosis* complex according to the currently accepted taxonomy (*M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. caprae*, *M. africanum*, *M. pinnipedii*, *M. microti*, *M. cannettii*), *M. bovis* and *M. caprae* were the most common isolates; *M. tuberculosis* was also occasionally isolated from tuberculous lesions from cattle in these Central European countries (Pavlik et al., 2002b,c, 2003a, 2005; Erler et al., 2004; Ocepek et al., 2005).

The purpose of the present study was to analyse the detection of bovine tuberculosis caused by *M. bovis* and *M. caprae* and human tuberculosis caused by *M. tuberculosis* and *M. africanum* in cattle and other animals in the regions of six Central European countries during 2000–2004.

MATERIAL AND METHODS

Characteristics of regions of the monitored countries

The study was performed in six countries of Central Europe lying between the Baltic and the Adriatic seas, covering an area of 610 402 km^2 with more than 68 million inhabitants. Until 2003, a total of 9 330 264 cattle had been kept in this area in 1 105 056 establishments (Table 1).

Sources of statistical data on the incidence of bovine tuberculosis in cattle

Data on the incidence of bovine tuberculosis in cattle and other animals was obtained from National Reference Laboratories for Bovine Tuberculosis in six Central European countries.

Intravital and postmortem diagnosis of bovine tuberculosis in cattle

Single intradermal testing with bovine tuberculin was performed in all cattle older than two years at least every second year during the monitored period. Animals with a positive response to single testing (bovine tuberculin only) were examined using the comparative test (bovine and avian tuberculin) at least 6 to 8 weeks later. Animals giving a second positive response to bovine tuberculin were slaughtered and their lymph nodes were examined by laboratory methods (histology, direct microscopy of homogenized tissue, and culture) in the respective National Reference Laboratories for Bovine Tuberculosis.

In Slovenia skin testing with bovine tuberculin was carried out throughout the study period in all cattle

Table 1. Data describing national control programmes against bovine tuberculosis in cattle

Country	National control programmes	Cattle husbandry[1]		Geographic data[2]	
		establishments	cattle (total in mil)	km^2	inhabitants (mil)
Croatia	1953–1966	84 546	0.398 037	56 538	4.505
Czech Republic	1959–1968	28 000	1.473 828	78 864	10.330
Hungary	1962–1980	32 721	0.766 000	93 031	10.160
Poland	1959–1975	898 464	5.652 164	312 683	35.735
Slovakia	1959–1968	15 123	0.590 382	49 035	5.350
Slovenia	1962–1973	46 202	0.449 853	20 251	1.950
Total	1953–1980	1 105 056	9.330 264	610 402	68.030

[1]official data from 2003 from OIE (2004)

[2]official data from 2003 from Index Mundi: http://www.indexmundi.com

older than six weeks that had been reared on milk producing farms in the years 2000–2003. In 2004, all cattle older than six weeks were skin tested.

Tuberculous lesions detected by conventional veterinary-meat inspection of slaughtered cattle and other animals were likewise examined for the presence of mycobacteria by previously described laboratory methods (Pavlik et al., 2002d). Mycobacterial isolates were identified biochemically and by biological experiments in guinea pigs (Wayne and Kubica, 1986). All the isolates were identified by probes i.e. AccuProbe (Gen-Probe Incorporated, San Diego, California, USA) and GenoType MTBC molecular assay (Hain Lifescience, Nehren, Germany).

RESULTS

During the 5-year-period, tuberculosis was not diagnosed in cattle in the Czech Republic and Slovakia. The incidence of bovine tuberculosis in cattle herds in the other countries ranged between 0% and 0.012%. Accordingly, these countries satisfied the OIE criteria for freedom from bovine tuberculosis in cattle during the study period (Table 2).

In the study area, bovine tuberculosis was diagnosed in a total of 188 herds in 1 547 cattle, predominantly in small herds of < 10 cows (77.1%). Between 2000 and 2003, the numbers of detected herds decreased from 49 to 26 infected cattle herds; however, bovine tuberculosis was diagnosed in 48 cattle herds in 2004. This was particularly due to outbreaks in Poland, where 44 cattle herds were diagnosed as infected (Table 2).

Bovine tuberculosis was diagnosed in 45 animals of 9 species other than cattle from five of the six countries (the exception being Slovakia). Infection was detected in 37 (82.2%) wild animals: 15 wild boars in Hungary and Croatia, 6 red deer in Hungary, and 14 European bison and 2 roe-deer in Poland. Bovine tuberculosis was detected in 8 (17.8%) animals of 5 species in zoological gardens (Table 3).

Two other members of *M. tuberculosis* complex, *M. africanum* and *M. tuberculosis*, were diagnosed in one animal from a zoological garden in Croatia and in nine domestic animals that came into contact with men (five head of cattle, two domestic pigs and two dogs), respectively. In Slovenia *M. tuberculosis* was isolated from one animal in a large cattle herd of 75 animals and in Poland from four cattle in small cattle herds of less than 9 cows (Table 4).

DISCUSSION

National control programmes against bovine tuberculosis in cattle had been implemented before 1980 (Table 1) in all six Central European countries investigated in the present study. No member of *M. tuberculosis* complex was detected in any of the animals in Slovakia (Tables 2, 3, and 4). However, as is evident from the results, cases of bovine tuberculosis are still occasionally diagnosed in cattle, other domestic animals and in wild animals in captivity or in the natural environment in the other studied countries (Tables 2 and 3). Most outbreaks of bovine tuberculosis in cattle were diagnosed in Poland (Table 2) in both small and large cattle herds. The

Table 2. Bovine tuberculosis in cattle herds in Central European countries

Country	No. of herds		Year					Total No.
	size	total No.[3]	2000	2001	2002	2003	2004	
Croatia	small[1]	N	8	10	4	3	2	27
	large[2]	N	0	0	0	0	1	1
	total	84 546	8	10	4	3	3	28
	%	100	0.010	0.012	0.005	0.004	0.004	
	cases		8	21	10	7	15	61
Czech Republic	total	28 000	0	0	0	0	0	0
	%	100	0	0	0	0	0	0
Hungary	small[1]	N	2	1	2	0	0	5
	large[2]	N	2	1	1	1	1	6
	total	32 721	4	2	3	1	1	11
	%	100	0.012	0.006	0.009	0.003	0.003	
	cases		5	3	4	18	5	35
Poland	small[1]	N	32	18	16	14	30	110
	large[2]	N	5	5	4	8	14	36
	total	898 464	37	23	20	22	44	146
	%	100	0.004	0.003	0.002	0.003	0.005	
	cases		174	156	255	309	554	1 448
Slovakia	total	15 123	0	0	0	0	0	0
	%	100	0	0	0	0	0	0
Slovenia	small[1]	N	0	1	2	0	0	3
	large[2]	N	0	0	0	0	0	0
	total	46 202	0	1	2	0	0	3
	%	N	0	0.002	0.004	0	0	
	cases		0	1	2	0	0	3
Total No. of	small[1]	N	42	30	24	17	32	145
	large[2]	N	7	6	5	9	16	43
	herds	1 225 411	49	36	29	26	48	188
	%	100	0.004	0.003	0.002	0.002	0.004	
	cases		187	181	271	334	574	1 547
	%		12.1	11.7	17.5	1.6	37.1	100

[1]small cattle herd (≤ 10 cows); [2]large cattle herd (> 10 cows); [3]No. of establishments in 2003 (OIE, 2004); N = official data not available

unexplained increase in incidence in 2004 (44 infected herds), that was even higher than in 2000 (only 37 infected herds), is a concern. In Hungary the economic impact of bovine tuberculosis may have been more significant than in other studied countries because 6 of 11 outbreaks were in large cattle herds (Table 2). Causative agents *M. bovis* and *M. caprae* were isolated from infected animals (Pavlik et al., 2002c; Lantos et al., 2003; Erler et al., 2004).

Table 3. Animal species other than cattle infected with bovine tuberculosis

Type of breed	No. (%)	Animal species	No.	Year	Country	Notes
Free nature	37 (82.2)	Wild boar (*Sus scrofa*); *n* = 15	3	2000	Hungary	Districts with BTB[1]
			6	2001	Hungary	Districts with BTB[1]
			2	2002	Hungary	Districts with BTB[1]
			1	2003	Hungary	Districts with BTB[1]
			2	2004	Hungary	Districts with BTB[1]
			1	2004	Croatia	Forestry Pozega*
		Red deer (*Cervus elaphus*); *n* = 6	5	2000	Hungary	National park*
			1	2002	Hungary	National park*
		European bison (*Bison bison* f. *bonasus*)[1]; *n* = 14	10	2000	Poland	District without BTB[2]*
			4	2001	Poland	District with BTB[1]
		Roe deer (Capreolus capreolus f. capreolus); *n* = 2	2	2004	Poland	District without BTB[2]*
Zoological gardens	8 (17.8)	Bactrian camel (*Camelus ferus*); *n* = 2	2	2002	Czech Republic	Prague[3]*
		Siberian Tiger (*Panthera tigris* f. *altaica*); *n* = 1	1	2002	Hungary	Budapest[4]*
		Bison (*Bison bison*); *n* = 3	1	2002	Poland	Gdansk*
			2	2004	Slovenia	Ljubljana*
		Dromedary camel (*Camelus dromedarius)*; *n* = 1	1	2004	Slovenia	Ljubljana*
		Eland (*Taurotragus oryx*); *n* = 1	1	2004	Poland	Gdansk*
Total	45 (100)		45			

[1]BTB = bovine tuberculosis was diagnosed in cattle or other domestic animals

[2]BTB = bovine tuberculosis was not diagnosed in cattle or other domestic animals

[3]male (16 years) and female (17 years) Bactrian camels were imported from Near East in 1985, isolates were previously identified by spoligotyping as *M. caprae* (Erler et al., 2004)

[4]isolate was previously identified by spoligotyping as *M. caprae* (Lantos et al., 2003)

*the source of infection not known

The outcomes of the successful national control programmes can be particularly seen in the Czech Republic and in Slovakia where bovine tuberculosis in cattle was not diagnosed during the study period (Table 2). The success of the Czech and Slovak control of bovine tuberculosis was achieved three decades after the completion of the programme in 1968 (Pavlik et al., 1998, 2002a,e). Regular yearly skin testing with bovine tuberculin has especially contributed to the control of the disease in domestic animals (Pavlik et al., 2002d, 2005). However, tuberculous lesions had been found in cattle and pigs in the Czech Republic between 1990 and 1999 (Pavlik et al., 2002d, 2003b; Vecerek et al., 2003; Kozak et al., 2004) and were still detected between 2000 and 2004 (Pavlik et al., 2005). In the last decade, besides the isolated causative agents *M. bovis*, *M. caprae* and *M. tuberculosis* particularly *M. avium* complex members, conditionally pathogenic mycobacteria and occasionally *M. a. paratuberculosis* were isolated (Dvorska et al., 1999, 2004; Pavlik et al., 2002d, 2003b; Machackova-Kopecna et al., 2005). *Rhodococcus equi* was isolated from some tuberculoid lesions of pigs and cattle either alone or together with conditionally pathogenic mycobacteria (Dvorska et al., 1999; Pavlik et al., 2002d; Pate et al., 2004).

An increased incidence of tuberculous lesions in pigs has been recorded in the Czech Republic in recent years; however, these were not caused by the

Table 4. *Mycobacterium tuberculosis* (n = 9) and *M. africanum* (n = 1) infection in animals

Country	Total No.	Year	Hyrax[1]	Cattle[2]	Pig[3]	Dog[4]	Total
Croatia	1	2004	1	0	0	0	1
Czech Republic	1	2004	0	0	0	1[5]	1
Hungary	1	2004	0	0	0	1[6]	1
		2000	0	1*	1	0	2
Poland	6	2001	0	2*	0	0	2
		2002	0	1*	1	0	2
Slovenia	1	2000	0	1**	0	0	1
Total	10	2000–2004	1	5	2	2	10

[1]hyrax (*Procaria habessinica*) infected with *Mycobacterium africanum* in zoological garden in Zagreb

[2]cattle (*Bos taurus*)

[3]domestic pig (*Sus scrofa* f. *domestica*)

[4]dog (*Canis lupus* f. *familiaris*)

[5]one female of Doberman breed (5.5 years) was infected in lungs and mesenteric lymph nodes (Trcka et al., 2005)

[6]male of West Highland White terrier (2.0 years) was infected in exudation from the thoracic cavity

*from a small cattle farm with less than 9 cows

**from a large cattle farm with 75 cows

members of *M. tuberculosis* complex. External environments, particularly those contaminated with the members of *M. avium* complex (Horvathova et al., 1997; Matlova et al., 2003), peat and kaolin used as feed supplements (Trckova et al., 2004; Matlova et al., 2004a, 2005) and deep litter containing enzymatically digested sawdust (Matlova et al., 2004b) were shown to pose a high risk to animals. After the replacement of sawdust with bran, it was found that bran was also susceptible to contamination with *M. avium* complex members as feeding bran to pigs caused tuberculous lesions in their lymph nodes (Fischer et al., 2003).

Most cases of bovine tuberculosis in the period 1990–1999 in Central Europe were animals from zoological gardens (Pavlik et al., 1998; 2002e). However, in the latest study period, the occurrence of bovine tuberculosis in wild animals, particularly wild boars, cervids (red deer and roe deer) and European bison in Poland, Croatia and Hungary (Table 3) may be epidemiologically significant. Since wild boar migrate over relatively large areas (Machackova et al., 2003) the detection of the causative agent of bovine tuberculosis in one wild boar in Croatia and in 14 wild boar in Hungary is concerning (Table 3).

Bovine tuberculosis has also been detected in other parts of Europe in wild boars, red deer (*Cervus elaphus*), fallow deer (*Dama dama*), Iberian lynx (*Lynx pardina*), and hare (*Lepus europaeus*) in Spain (Aranaz et al., 2004). During the study period in these six central European countries bovine tuberculosis transmission from wild animals to domestic was never documented. On the other hand, it is concerning that bovine tuberculosis was detected in free living animals in Poland, both in districts with tuberculosis-infected domestic animals (particularly cattle) and in districts with infection-free domestic animals (Tables 2 and 3).

Bovine tuberculosis was also diagnosed in the strictly protected European bison in Poland both in the previously analysed period between 1990 and 1999 (Pavlik et al., 2002e) and in recent years (2000 and 2004; Table 3). This is a concern as some of these European bison were in the same region as the bovine tuberculosis-free cattle population (Table 3; Pavlik et al., 2002e).

The potential role of wild animals in the spread of *M. bovis* infection in domestic livestock is of particular importance in countries where eradication programs have substantially reduced the incidence of bovine tuberculosis but sporadic outbreaks still occur. The best-known examples are the European badger (*Meles meles*) in the United Kingdom and the Republic of Ireland (Little et al., 1982; Gallagher and Clifton-Hadley, 2000) and the Australian brush-tailed possum (*Trichosurus vulpecula*) in New Zealand (Coleman and Cooke, 2001). The po-

tential for a badger population to become endemically infected with *M. bovis* and to act as a source of infection for cattle has been proven experimentally (Little et al., 1982). The involvement of badgers and possums has been based principally on the observed incidence of infection in these animals inhabiting affected areas, coupled with the finding that intervention studies that removed badgers or possums were shown to result in a consequential decrease in the number of tuberculosis-infected cattle and re-infections (O'Mairtin et al., 1998; Gallagher and Clifton-Hadley, 2000).

The causal agent of human tuberculosis can be isolated from companion dogs (Table 4) which could presumably have been infected by man. In the Czech Republic the isolate of the same spoligotype and IS*6110* RFLP type was obtained from a middle aged man who suffered from tuberculosis and later died of pulmonary tuberculosis as well as his dog (Trcka et al., 2005).

Further epidemiological studies and molecular typing of *M. bovis* and/or *M. caprae* isolates from domestic and wild animals originating from these central European countries are required for conclusion that the infections in some or all of these European wild species may be a dead-end spill-over from cattle.

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