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Incidence of bovine tuberculosis in wild and domestic animals other than cattle in six Central European countries during 1990–1999

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Incidence of bovine tuberculosis in wild and domestic animals other than cattle in six Central European countries during 1990–1999

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ABSTRACT: The study was undertaken in Croatia, Czech Republic, Hungary, Poland, Slovakia and Slovenia laying between Baltic and Adriatic seas on 610 402 km^2. *Mycobacterium bovis* infection was diagnosed in 70 animals belonging to 17 species other than cattle. The set of wild animals comprised 12 European bison (*Bison bonasus*), one red deer (*Cervus elaphus*), five wild boars (*Sus scrofa*), and one European wild goat (*Capra aegagrus*) bred in a game park. Further positive animals included two farmed red deer (*Cervus elaphus*) and one bactrian camel (*Camelus ferus*) owned by a circus. The infection was also demonstrated in 18 domestic animals belonging to 3 species living on farms where bovine tuberculosis was diagnosed in cattle. This set included 12 domestic pigs (*Sus scrofa* f. *domestica*), two domestic sheep (*Ovis ammon* f. *aries*), and four dogs (*Canis lupus* f. *familiaris*). The set of animals bred in zoological gardens consisted of 30 animals belonging to 9 species as follows: three bison (*Bison bison*), four tapirs (*Tapirus terrestris*), one cassowary (*Casuarius casuarius* – isolate identified by the biological assay in guinea pigs only), eight sitatungas (*Tragelaphus spekei*), three elands (*Taurotragus oryx*), one gnu (*Connochaetes taurinus*), eight reticulated giraffes (*Giraffa cameloparadlis reticulata*), one puma (*Puma concolor*), and one Vietnamese pot-bellied pig (*Sus bucculentus*). Although, considering the population sizes, absolute numbers of the infected individuals are rather low, wild animals or such animals bred in captivity should be regarded as possible reservoirs of the causative agent of bovine tuberculosis. Tests for bovine tuberculosis are therefore necessary before transportation of all wild animals. Any lesion arousing suspicion of tuberculosis found on necropsy of wild animals must be laboratory examined for the presence of mycobacteria.

Keywords: *Mycobacterium bovis*; veterinary epidemiology; zoological gardens; game parks; farmed deer; wild animals

National programmes for the elimination of *Mycobacterium bovis* infection in cattle were successfully completed in seven Central European countries (Bosnia and Herzegovina, Croatia, Czech Republic, Hungary, Poland, Slovakia, and Slovenia) in the second half of the 20th century (Table 1). In the subsequent post-elimination period, the incidence of bovine tuberculosis in cattle decreased and reached zero level in some countries, or sporadic outbreaks of bovine tuberculosis in cattle were recorded in Slovakia and Slovenia in 1993, in the Czech Republic in 1995, in Croatia and Bosnia and Herzegovina in 1998 (Pavlik *et al.*, 2002; Table 1).

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The elimination of the major source of *M. bovis* was accompanied by decreasing incidence of this infection in other animal species in Croatia (Kovacic *et al.*, 1998), the Czech Republic (Polak, 1969; Kouba, 1988; Pavlik *et al.*, 1998; Pavlas, 1999), Hungary (Jamniczky *et al.*, 1995; Balint, 1995; Körmendy, 1995), Poland (Lis, 1998; Zorawski and Lipiec, 1998b), Slovakia (Tanuska, 1982; Vasilova, 1990; Hanzlikova and Vilimek, 1992), and Slovenia (Prevocnik and Ocepek, 1994). Complete eradication of bovine tuberculosis has not been achieved due to the long incubation period of the disease. In some countries, such as the Czech Republic, infections by *M. bovis* were most frequently diagnosed in animals bred in zoological gardens (Pavlik *et a*l., 1998). The most frequent sources of *M. bovis* are infected animals of different origin which can relatively quickly spread the causal agent in zoological gardens. Further possible sources of *M. bovis* are infected attendants and/or visitors (Thoen *et al.*, 1995).

Bovine tuberculosis was diagnosed in the continental Europe also in wild animals. An outbreak affecting 15 red deer (*Cervus elaphus*) was recorded in northeastern Bohemia in the beginning of 20th century (Herkner, 1913). Further cases were diagnosed in roe deer (*Capreolus capreolus*) (Krul, 1962) and wild boars (*Sus scrofa scrofa*) in Slovakia (Hanzlikova and Vilimek, 1992; Kalensky, 1992), Germany (Schulz *et al.*, 1992), and Italy (Mignone *et al.*, 1991; Biolatti *et al.*, 1992; Serraino *et al.*, 1999). Bovine tuberculosis poses a serious economic and health problem also in farmed cervids. Infections by *M. bovis* were reported from other countries like Denmark (Jorgensen *et al.*, 1988) and Sweden (Bölske *et al.*, 1995).

Although less known, other wild animal species can also become important sources of *M. bovis* infections for domesticated animals. For example infected badgers (*Meles meles*) were identified as reservoirs and vectors of bovine tuberculosis in the United Kingdom (Cheeseman *et al.*, 1985, 1989) and in Ireland (Grange *et al.*, 1990; McAleer, 1990). Infection by *M. bovis* was also diagnosed in possums (*Trichosurus vulpecula*) in New Zealand (Lugton *et al.*, 1997) and in sika deer (*Cervus nippon*) bred in captivity in the United States (Mirsky *et al.*, 1992). Under specific epidemiological conditions such animals could have been sources of infection for farm animals in areas from which infection in cattle had been eradicated. Bovine tuberculosis can be transmitted also among animals living in the wild. A case of coyotes (*Canis latrans*) infected by ingestion of dead white-tailed deer (*Odocoileus virginianus*) living in the wild in an area infected by bovine tuberculosis was reported from Michigan, USA (Bruning-Fann *et al.*, 1998). In Great Britain *M. bovis* was isolated from rats (*Rattus norvegicus*) and foxes (*Vulpes vulpes*) (Little *et al.*, 1982). In Kruger National Park (South Africa) *M. bovis* was isolated from African buffalo (*Syncerus caffer*), chacma baboon (*Papio ursinus*), lion (*Panthera leo*), cheetah (*Acinonyx jubatus*), kudu (*Tragelaphus strepsiceros*) and leopard (*Panthera pardus*) (De Vos *et al.*, 2001).

These facts can be understood as a precaution that the current extremely low incidence of bovine tuberculosis in domestic ruminants in Central European countries can dramatically change under the influence of unknown risk factors. The objective of this study was to analyse the incidence of bovine tuberculosis in animal species other than cattle in Central Europe from 1990 to 1999 and to assess the hazard of transmission of *M. bovis* within and between the populations of domestic and wild animal species.

MATERIAL AND METHODS

Characteristics of the countries under study

The investigations were conducted in six Central European countries, including Croatia, the Czech Republic, Hungary, Poland, Slovakia, and Slovenia, situated between the Baltic and the Adriatic seas and covering an area of 610 402 km^2. Until 1995, a total of 12 790 million heads of cattle were bred in this area, of which 5.901 millions were cows (FAO-OIE-WHO, 1997; OIE, 1999, 2000). According to a WHO (http://who.int) source, the human population reached 68 millions of inhabitants (Table 1).

At the beginning of the investigation period, grazing of cattle was more practised in Poland and in highland and foothill areas of Slovakia and Slovenia. In the last ten years the number of farmers preferring grazing practice has been increasing also in the Czech Republic, Hungary, and Croatia.

Sources of statistical data on the incidence of bovine tuberculosis

Data on the incidence of bovine tuberculosis in 1990 through 1999 were obtained from National Reference Laboratories for bovine tuberculosis of all the six countries. Each laboratory has provided verified anamnestic data on the species and origin of the affected animals and on possible contacts with other infected animals including cattle (Table 2).

Table 1. Basic data describing Central European countries presented in paper

Country	National control programmes against *M. bovis* in cattle	Number of								
		cattle (mil.)	cows (mil.)	establish-ments	cattle out-breaks during 1990–1999**	sheep (mil.)	goats (mil.)	pigs (mil.)	area (km^2)	inhabitants (mil.)
Croatia	1953–1966	0.493	0.330	n	4 (1993)	0.426***	0.150***	1.166***	56 538	4.505
Czech Republic	1959–1968	2.030	0.830	5 410	7 (1995)	0.200	0.045	3.867	78 864	10.330
Hungary	1962–1980	0.910	0.420	50 936	51	0.947	0.052	4.356	93 031	10.160
Poland	1959–1975	7.937	3.763	1 373 500	980	0.800	0.177***	20.500	312 683	35.735
Slovakia	1959–1968	0.916	0.348	1 369	8 (1993)	0.397	0.012	2.037	49 035	5.350
Slovenia	1962–1973	0.504	0.210	n	32 (1998)	0.020	0.010	0.592	20 251	1.950
Total	1953–1980	12.790	5.901		975	2.790	1.319	32.518	610 402	68.030

Explanations:

*geographic, demographic and agricultural data from: WHO (http://who.int), Surveillance of Tuberculosis in Europe (http://www.ceses.org/eurotb/eurotb.htm) and OIE (FAO-OIE-WHO, 1997, Animal Health Yearbook, Rome, Italy, 280 pp.) from 1995

**data published by Pavlik *et al.* (2002)

***data from 1997 (OIE, 1998)

(1993, 1995, 1998) – last outbreak of bovine tuberculosis in cattle

n – official data not available

Table 2. Animal species infected with *Mycobacterium bovis*

Type of breed	Number	Animals species		Number	Year	Country	Notes
Free nature	18 25.7%	Wild boar (*Sus scrofa scrofa*)	*n* = 5	3	1992	Slovakia	district with BTB[2]
				1	1992	Croatia	n
				1	1996	Hungary	district with BTB[2]
		Red deer (*Cervus elaphus*)	*n* = 1	1	1991	Czech Republic	n
		European bison (*Bison bonasus*)[1]	*n* = 12	3	1997	Poland	district with BTB[2]
				7	1998		
				2	1999		
Game park	1 1.4%	European wild goat (*Capra aegagrus*)[4]	*n* = 1	1	1991	Czech Republic	n
Circus	1 1.4%	Bactrian camel (*Camelus ferus*)	*n* = 1	1	1992	Czech Republic	n
Zoological gardens	30 42.9%	Bison (*Bison bison*)	*n* = 3	1	1993	Czech Republic	Prague
				2	1996	Poland	Gdansk
		Tapir (*Tapirus terrestris*)	*n* = 4	1	1994	Czech Republic	Jihlava[3]
				2	1995		
		Sitatunga (*Tragelaphus spekei*)	*n* = 8	1	1997	Poland	Poznan
				4	1991		Wroclaw
		Elands (*Taurotragus oryx*)	*n* = 3	1	1992		
				4	1993		
		Gnu (*Connochaetes taurinus*)	*n* = 1	1	1994		
				2	1995		
		Reticulated giraffe (*Giraffa cameloparadlis reticulata*)	*n* = 8	4	1991	Poland	Wroclaw
				3	1994		
				1	1995		
		Vietnamese pot-bellied pig (*Sus bucculentus*)	*n* = 1	1	1995	Poland	Wroclaw
		Puma (*Puma concolor*)	*n* = 1	1	1999	Hungary	Budapest
		Cassowary (*Casuarius casuarius*)[5]	*n* = 1	1	1994	Czech Republic	Brno
Cervid farm	2 2.9%	Red deer (*Cervus elaphus)*	*n* = 2	1	1999	Czech Republic	n
				1	1999	Hungary	n
Cattle farm	18 25.7%	Domestic pig (*Sus scrofa* f. *domestica*)	*n* = 12	2	1990	Poland	All animals in contact with infected cattle herds with *M. bovis*
				5	1995		
				1	1996	Poland	
				4	1997		
		Domestic sheep (*Ovis ammon* f. *aries*)	*n* = 2	1	1990	Poland	
				1	1997		
		Dog (*Canis lupus* f. *familiaris*)	*n* = 1	4	1997	Poland	
Total	70 100%			70			

Explanations:
[1]wild European bison from the protected area in Bieszczady mountains (Poland)
[2]district with bovine tuberculosis in cattle in the pasture
[3]tapirs imported to zoological garden in Jihlava (the Czech Republic) from zoological garden in Poznan (Poland) in 1994
[4]histological examination only
[5]isolate identified by the biological trial on guinea pig only
n – official data not available

Post-mortem diagnosis of bovine tuberculosis

Lesions indicative of tuberculosis found on necropsy during the monitoring period were subjected to laboratory examination. In all the six countries, the examinations were conducted by the National Reference Laboratories or laboratories authorised by the respective state veterinary authority.

Tissue samples were processed by standard histological procedures including fixation with formalin and staining with haematoxylin-eosin and Ziehl-Neelsen (Fischer *et al.*, 1999). Fresh or frozen samples (approx. 1 g) were homogenised and examined for the presence of mycobacteria by direct microscopy after staining with Ziehl-Neelsen and by culture (Fischer *et al.*, 2000).

Most of the isolates were classified with the *M. tuberculosis* complex using the Accu-probes (Gen-Probe Incorporated, San Diego, California, USA) and subsequently identified by biochemical methods and bioassays in guinea pigs (Wayne and Kubica, 1986).

RESULTS

The total number of animals other than cattle in which infection by *M. bovis* was diagnosed during the period of investigation was 70 (Table 3). Most of the cases (70%) were reported from Poland. On the other hand, no cases of bovine tuberculosis in animals other than cattle were reported from Slovenia and since 1992 also from Slovakia and Croatia. The lowest and the highest proportions of the 70 infected animals were recorded in 1990 (4.3%) and 1997 (18.6%), respectively. Most cases were diagnosed in zoological gardens (42.9%), in the wild (25.7%), and in animals bred on cattle farms infected by bovine tuberculosis (25.7%). The incidence was markedly lower in farmed red deer (2.9%), animals living in game parks (1.4%) and in circus (1.4%) (Table 3).

M. bovis was diagnosed in 17 animal species (Table 2). Those living **in the wild** included 18 animals belonging to three species: 12 European bison (*Bison bonasus*), one red deer, and five wild boars. Only one case in an European wild goat (*Capra aegagrus*), two cases in red deer, and one case in a bactrian camel (*Camelus ferus*) were diagnosed in **game parks**, **red deer farms**, and **circus**, respectively. Infections by *M. bovis* were demonstrated in 18 animals bred on **cattle farms affected by bovine tuberculosis**; the set included 12 domestic pigs (*Sus scrofa* f. *domestica*), two domestic sheep (*Ovis ammon* f. *aries*), and four dogs (*Canis lupus* f. *familiaris*).

Bovine tuberculosis was diagnosed in seven **zoological gardens** in three countries (the Czech Republic, Hungary, and Poland). The 30 infected animals belonged to nine species as follows: three bison (*Bison bison*), four tapirs (*Tapirus terrestris*), one cassowary (*Casuarius casuarius*) – isolate identified by the biological trial on guinea pig only, eight sitatungas (*Tragelaphus spekei*), three elands (*Taurotragus oryx*), one gnu (*Connochaetes taurinus*), eight reticulated giraffes (*Giraffa cameloparadlis reticulata*), one puma (*Puma concolor*), and one Vietnamese pot-bellied pig (*Sus bucculentus*). Moreover, *M. bovis* infection was diagnosed in quarantine of the zoological garden in Jihlava (the Czech Republic) and in tapirs imported from Poznan – Poland (Table 2).

DISCUSSION

National control programmes against bovine tuberculosis in cattle were completed in all the six Central European countries included in our study (Pavlik *et al.*, 2002). Their success are documented by a relatively low incidence of bovine tuberculosis in cattle in Poland and Hungary in 1990 through 1999 and by the last cases of *M. bovis* isolated from cattle in Croatia, the Czech Republic, Slovakia and Slovenia, during this period (Pavlik *et al.*, 2002; Table 3). In the subsequent long post-elimination period (such as in the Czech Republic) the incidence of bovine tuberculosis decreased not only in cattle, but also in other animal species of domestic and wild animals (Pavlik *et al.*, 1998).

Host spectrum

The rather diverse species composition of the study of animals is indicative of a wide host spectrum of *M. bovis* (Table 2). It is therefore necessary in the post-elimination period to include into laboratory examination tissue samples suspected of tuberculosis collected not only from cattle, but also from animals living in the wild or in captivity (Table 2). The danger of transmission to other animal species by direct or indirect contact must be considered in the case of new outbreaks of bovine tuberculosis in cattle. Bovine tuberculosis is transmissible to man who can become source of infection for animals (Schliesser, 1974; Englert and Milbradt, 1977; Hejlicek and Chloupek, 1982; Pavlas and Mezensky, 1982; Pavlik *et al.*, 1998).

Animals in the wild

The incidence of bovine tuberculosis in wild ruminants in countries where it has been eliminated in cattle and other animal species is sporadic. The first case of bovine tuberculosis in a wild red deer in New Zealand was diagnosed during the late 1970s (DeLisle *et al.*, 1993). Further cases of bovine tuberculosis in cloven hoofed game were reported from Ireland (Dodd, 1984), USA (Miller *et al.*, 1997; Schmitt *et al.*, 1997), and Great Britain (Gunning, 1985; Clifton-Hadley and Wilesmith 1991). In our investigations, *M. bovis* was detected only in one red deer living in the wild in the Czech Republic and in three, one, and one wild boar in Slovakia, Croatia, and Hungary, respectively (Table 2).

A rather specific problem was an outbreak of bovine tuberculosis in a herd of European bison living in the wild in the area of Bieszczady (Poland) (Table 2). The first case of *M. bovis* infection was diagnosed in 1997 (Zorawski and Lipiec, 1997, 1998a) and further cases were detected repeatedly in the subsequent years (un published data). The probable source was *M. bovis*-infected cattle bred in this area. One of the infected cattle farms was located adjacent to the area in which a herd of approximately 30 European bison lived (Zorawski and Lipiec, 1997). The prevalence of infection was rather high and it was therefore decided to kill the whole bison's population in this area.

Active screening for sources of *M. bovis*, preferably by tuberculin skin test of cattle older than 2 years, is necessary to prevent the transmission of *M. bovis* to wild animals. This measure is particularly important in cattle herds in which bovine tuberculosis has been diagnosed recently.

Animals in game parks

Parks for cloven-hoofed game pose a quite specific epizootiological problem. High concentration of animals in a limited area is associated with a risk of spreading of *M. bovis* within the park once the agent has been introduced thereto. Published data indicate that bovine tuberculosis has been a rare health problem of cloven-hoofed animals bred in game parks, although such cases were reported from Ireland (Quigly *et al.*, 1997), USA (Mirsky *et al.*, 1992), and Canada (Tessaro, 1986). Our set collected from six countries included only a single European wild goat found in the Czech Republic, in which tuberculosis was diagnosed by histological examination of liver samples in 1991 (Pavlik *et al.*, 1998; Table 2). Transmission of *M. bovis* from an infected herd of bison to red deer, moose (*Alces alces*), and white-tailed deer was reported from Canada (Tessaro, 1986).

The infection pressure in game parks is not apparently high as in zoological gardens or in deer farms, probably because living conditions approach those prevailing in the wild. Bovine tuberculosis can be transmitted to groups of wild ruminants breed in game parks from purchased animals or those transported from another environment, or by penetration into the park. Possible sources in such cases are wild boars, badgers, and foxes. It is therefore necessary to replace game park populations with wild animals caught in areas that are free of bovine tuberculosis, or with those reared in captivity in *M. bovis*-free herds (Thoen *et al.*, 1995). Protection against penetration of infected wild animals into the game park can be performed by firm fencing and its maintenance (Machackova *et al.*, 2001).

Farmed cervids

Spread of *M. bovis* among farmed cervids can be much more higher as compared to game parks as a result of a high concentration of animals on a limited area, frequent handling and other stressful conditions. In Great Britain *M. bovis* infection was diagnosed in 26 of the 106 red deer imported from Hungary (Stuart *et al.*, 1988). Another example was the introduction of *M. bovis* by fallow deer *(Dama dama*) imported from the United Kingdom first to one and subsequently to nine farms in Sweden (Bölske *et al.*, 1995). Transmission of bovine tuberculosis to a herd of bison sharing a pasture with farmed red deer was described in USA (Stumpf *et al.*, 1984).

Possible sources of infection for farmed cervids are infected new arrivals, infected wild animals penetrating into the farm, and under certain circumstances, *M. bovis*-infected farm personnel coming into contact with the farmed animals during feeding and handling (Thoen *et al.*, 1995).

Zoo animals

Sources and ways of transmission of *M. bovis* in zoo animals are practically the same as in farmed cervids. However, zoological gardens concentrate in a limited area many animal species coming from various countries and continents, and with different epizootiological

Table 3. Incidence of bovine tuberculosis in other animals than cattle in Central European countries

Country	Animals from	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	Total	%
Croatia	free nature	0	0	1	0	0	0	0	0	0	0	1	100.0
	Subtotal	0	0	1	0	0	0	0	0	0	0	1	100.0
Czech Republic	free nature	0	1	0	0	0	0	0	0	0	0	1	7.1
	game park	0	1	0	0	0	0	0	0	0	0	1	7.1
	zoological garden	0	0	0	1	2	2	0	0	0	0	5	35.8
	cattle farm	0	0	0	0	0	5	0	0	0	0	5	35.8
	cervid farm	0	0	0	0	0	0	0	0	0	1	1	7.1
	circus	0	0	1	0	0	0	0	0	0	0	1	7.1
	Subtotal	0	2	1	1	2	7	0	0	0	1	14	100.0
Hungary	free nature	0	0	0	0	0	0	1	0	0	0	1	33.3
	zoological garden	0	0	0	0	0	0	0	0	0	1	1	33.3
	cervid farm	0	0	0	0	0	0	0	0	0	1	1	33.4
	Subtotal	0	0	0	0	0	0	1	0	0	2	3	100.0
Poland	free nature	0	0	0	0	0	0	0	3	7	2	12	24.5
	zoological garden	0	8	1	4	4	4	2	1	0	0	24	49.0
	cattle farm	3	0	0	0	0	0	1	9	0	0	13	26.5
	Subtotal	3	8	1	4	4	4	3	13	7	2	49	100.0
Slovakia	free nature	0	0	3	0	0	0	0	0	0	0	3	100.0
	Subtotal	0	0	3	0	0	0	0	0	0	0	3	100.0
Slovenia	Subtotal	0	0	0	0	0	0	0	0	0	0	0	0.0
Subtotal	free nature	0	1	4	0	0	0	1	3	7	2	18	25.7
	zoological garden	0	8	1	5	6	6	2	1	0	1	30	42.9
	cattle farm	3	0	0	0	0	5	1	9	0	0	18	25.7
	cervid farm	0	0	0	0	0	0	0	0	0	2	2	2.9
	game park	0	1	0	0	0	0	0	0	0	0	1	1.4
	circus	0	0	1	0	0	0	0	0	0	0	1	1.4
Total number of animals		3	10	6	5	6	11	4	13	7	5	70	100.0
%		4.3	14.3	8.6	7.1	8.6	15.7	5.7	18.6	10.0	7.1	100	

histories (Thoen *et al.*, 1995). Therefore, the hazard of spread of infection in zoological gardens, once *M. bovis* has been introduced is very serious. Reports of repeated findings of *M. bovis* are evidence in Poland and Czech zoological gardens (Table 2). Another risk factor is the rather long incubation period (up to several years) of bovine tuberculosis, which was most probably responsible for the import of infected tapirs from the Poznan (Poland) to Jihlava (the Czech Republic) zoological garden. *M. bovis* was identified in one of three tapirs soon after the stressful transport in the autumn 1994 and in another two in the spring 1995. Remarkably, the first infection in the herd in Poznan was diagnosed as late as in 1997, but anamnestic data and results of examinations indicate that *M. bovis* had not been identified in the zoological garden Jihlava (the Czech Republic) from 1975 (Pavlik *et al.*, 1998; Table 2). The only explanation is that the animals had been infected before the stressful transport.

Circus

Further relevant factor for the spread of bovine tuberculosis includes the long life span of animals in zoological gardens and different sources of origin. Another risk factor is presented by the possible transmission of the causal agent of bovine tuberculosis from zoo attendants or visitors who may be shedders of *M. bovis* to bred animals (Thoen *et al.*, 1995).

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