Mycobacterium tuberculosis in animal and human populations in six Central European countries during 1990–1999

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ABSTRACT: Results of *Mycobacterium tuberculosis* detection in animals from six Central European countries (Croatia, the Czech Republic, Hungary, Poland, Slovakia and Slovenia) spreading over 610 402 km^2 with a population of 11.8 million heads of cattle were analysed. In the monitoring period, 1990 to 1999, *M. tuberculosis* from animals was isolated only in two countries (Poland and Slovak Republic) from 16 animals with tuberculous lesions. These comprise nine heads of cattle (*Bos taurus*), four domestic pigs (*Sus scrofa* f. *domestica*) and three wild animals, an African elephant (*Loxodonta africana*), agouti (*Dasyprocta aguti*) and terrestrial tapir (*Tapirus terrestris*), originated form a zoological garden Gdansk in Poland. Steady decrease in the incidence of tuberculosis in humans was recorded during the monitoring period in all countries. The human population of the study countries was 68.03 million. In the period monitored, infection caused by *M. tuberculosis* was identified in a total of 241 040 patients with a decreasing incidence of tuberculosis found in all countries. The lowest relative bacteriologically confirmed disease was found in the Czech Republic, Slovak Republic and Slovenia. Given the low number of infected domestic and wild animals, the epidemiological and epizootiological situation may be considered auspicious.

Keywords: *Mycobacterium bovis*; human tuberculosis; risk assessment

In the majority the Central European countries, the incidence of bovine tuberculosis in animals was auspicious during the last decades (Thoen and Steele, 1995). The prevalence and incidence of *Mycobacterium bovis* in cattle in these countries was nearly zero (Pavlik *et al.*, 2002a,e). Similarly,

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this promising epidemiological condition of the disease was observed in other domestic animals (Pavlik *et al.*, 2002d). In wild animals, however, the infectious situation has developed differently in the last three decades depending on the conditions in which animals were living. In animals kept in captivity, mainly in zoological gardens, the incidence and prevalence of *M. bovis* decreased gradually (Pavlik *et al.*, 1998). The epidemiological situation of animals in wild has been unknown for a long time as a result of insufficient analysis of the population. In farmed deer bovine tuberculosis occurs only sporadically (Pavlik *et al.*, 2002b,d).

In wild animals kept on farms, e.g. red deer (*Cervus elaphus*), *M. bovis* was detected when bovine tuberculosis had already been controlled in domestic animals and animals kept in zoological gardens. In the Czech Republic, for example an incidence of bovine tuberculosis has been recorded in one deer farm in 1999 (Pavlik *et al.*, 2002b,d). In the rest of the study countries, however *M. bovis* was identified on several farms (Pavlik *et al.*, 2002d). The infectious situation in the wild in most central European countries is unknown because, apart from the Czech Republic, no monitoring of the occurrence of bovine tuberculosis in wild animals has been conducted as yet. Nevertheless, *M. bovis* has been identified in animals living in the wild during the past two decades in the wild pig (*Sus scrofa ferus*) in Bosnia and Herzegovina, Hungary, Croatia and Slovakia (Ivetic and Sudaric, 1987; Hanzlikova and Vilimek, 1992; Kalensky, 1992; Pavlik *et al.*, 2002d; Machackova *et al.*, 2003).

Of other wild animals, *M. bovis* has been identified from the European bison (*Bison bonasus*) in Poland, which most probably were infected from an infected cattle kept on pasture (Zorawski and Lipiec, 1997, 1998; Pavlik *et al.*, 2002d). In the Czech Republic *M. bovis* was isolated from the red deer living in the wild in 1991 (Pavlik *et al.*, 1998, 2002d). On the basis of the genomic DNA analysis of *M. bovis* isolates in the Czech Republic and Slovak Republic, a unique spoligotype was observed. This isolate was a unique spoligotype as compared with the results of more than 3 000 other *M. bovis* isolates examined using the same method, which are in the RIVM database in Netherlands, coming from Europe and other continents (Pavlik *et al.*, 2002b). From these results it can be inferred that as yet unidentified reservoirs of *M. bovis* may evidently exist in the wild.

However, *M. tuberculosis* (one of the *M. tuberculosis* complex organism) was also isolated from organs with tuberculous lesions in domestic and wild animals. For example, in Norway between 1970 and 1975, *M. tuberculosis* was isolated from 3 (0.1%) of 3 004 pigs examined (Flesja *et al.*, 1978). In England over 16 years (1953–1968) *M. tuberculosis* was isolated from 67 (10.7%) of 626 pigs and from 13 (0.09%) of 14 439 head of cattle examined (Lesslie and Birn, 1970). In the USA Thoen *et al.* (1981) identified *M. tuberculosis* in one (0.01%) animal between 1973 and 1977 while examining 7 007 heads of cattle. In 474 exotic animals, *M. tuberculosis* was identified in 29 (6.1%) non-human primates and wild ruminants. The highest positive cases were detected while examining 135 non-human primates of which *M. tuberculosis* was identified in 21 (15.6%). In contrast, *M. tuberculosis* was not identified during the examination of 2 036 domestic pigs.

In the Federal Republic of Germany *M. tuberculosis* was isolated between 1961 and 1970 in two (0.2%) of 1 135 head of cattle and in 10 (0.49%) of 2 055 pigs (Schliesser, 1976). In Slovakia *M. tuberculosis* was isolated in 1970 in 1 (0.18%) of 553 head of cattle (Popluhar *et al.*, 1970), in 1972 in two (0.03%) of 6 684 head of cattle and in one (0.07%) of 1 421 pigs (Popluhar *et al.*, 1974). In the Czech Republic *M. tuberculosis* was isolated in 1984 from the tuberculosis-changed lungs of one old dog whose owner was treated for lung tuberculosis caused by the *M. tuberculosis* (Cada, 1994; Stika, 1994). The *M. tuberculosis* infection was also identified in dogs in Slovakia at the beginning of eradication programme against bovine tuberculosis (Canda, 1962).

The source of *M. tuberculosis* is most frequently considered to be a human being with tuberculosis, expelling the causal agent of human tuberculosis via sputum, less often through urine or faeces (Thoen and Steele, 1995; Grange, 1996). In Slovakia *M. tuberculosis* was isolated from stream water into which waste water flowed without adequate disinfection (Laktis *et al.*, 1970). Water supplies for animals contaminated in this way could also under certain circumstances become a source of infection either in barn or pasture.

In evaluating the results of examination of the presence of *M. tuberculosis* on the territory of some countries of Central Europe between 1990 and 1999, the question therefore arises as to what was the source of *M. tuberculosis* in domestic and wild animals.

MATERIAL AND METHODS

The characteristics of the territory in the countries monitored

The study was conducted on the territory of six countries of Central Europe (Croatia, Czech Republic, Hungary, Poland, Slovakia and Slovenia) lying between the Baltic and the Adriatic seas and covering an area of 610 402 km^2. According to data from the WHO (http://who.int) in 1995, more than 68 million inhabitants lived on the territory of these six countries. Until 1995, a total of 11.88 million heads of cattle have been kept in this area, of which more than 5.9 million were cows (FAO-OIE-WHO, 1997; Table 1).

The source of statistical data about the frequency of *M. tuberculosis* in human beings and animals

Data about the frequency of *M. tuberculosis* were obtained for the whole period between 1990 and 1999 from the relevant National Reference Laboratories. The tuberculosis-changed tissue of animals was examined for the presence of mycobacteria, which were identified as *M. tuberculosis* by biochemical tests and through biological experiment on guinea pigs (Wayne and Kubica, 1986).

RESULTS

The occurrence of *M. tuberculosis* in human beings in the years 1990 to 1999

In the period monitored bacteriologically confirmed tuberculosis caused by *M. tuberculosis* was identified in a total of 241 040 patients. In the period monitored, a decreasing frequency of tuberculosis was found in all countries. The lowest relative rate of bacteriologically confirmed cases of *M. tuberculosis* was found in the Czech Republic, Slovakia and Slovenia (Table 2).

The occurrence of *M. tuberculosis* in animals in the years 1990 to 1999

In the years 1990 to 1999, *M. tuberculosis* was isolated from the tuberculous lesions of 16 animals of 5 species in only two of the six countries monitored (Table 3).

The occurrence of *M. tuberculosis* in domestic animals in the years 1990 to 1999

In **Poland** *M. tuberculosis* was isolated from 9 heads of 9 cattle herds and from one domestic pig (Table 3). Each head of infected cattle originated

Table 1. Geographic, demographic and agricultural data about countries studied*

Country	Number of		Number of		
	inhabitants (mil.)	km^2	cattle (mil.)	cows (mil.)	herds
Croatia	4.505	56 538	0.493	0.330	nk
Czech Republic	10.330	78 864	2.030	0.830	5 410
Hungary	10.160	93 031	0.910	0.420	50 936
Poland	35.735	312 683	7.937	3.763	1 373 500
Slovakia	5.350	49 035	0.916	0.348	1 369
Slovenia	1.950	20 251	0.504	0.210	nk
Total	68.030	610 402	11.880	5.901	nk

Explanations:
*Source of the data: WHO (http://who.int), Surveillance of Tuberculosis in Europe (http://www.ceses.org/euroth/eurotb.htm) and OIE (FAO-OIE-WHO, 1997) from 1995
nk = not known

Table 2. Bacteriologically confirmed cases of *M. tuberculosis* infection in human population during 1990–1999

Year	Croatia		Czech Republic		Hungary		Poland		Slovakia		Slovenia		Total
	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.
1990	2 576	55.0	1 505	14.5	3 588	34.0	16 136	42.3	1 191	22.7	419	22.1	25 415
1991	2 158	45.0	1 549	14.9	3 568	35.0	16 497	43.1	1 197	22.7	363	19.1	25 332
1992	2 185	46.0	1 420	13.8	3 960	38.0	16 551	43.1	1 180	22.6	388	20.4	25 684
1993	2 279	48.0	1 222	11.8	4 209	41.0	16 828	43.8	1 139	22.4	420	22.1	26 097
1994	2 217	46.0	1 161	11.2	4 163	41.0	16 653	43.2	1 165	22.5	334	17.6	25 693
1995	2 114	44.0	1 188	11.5	4 339	42.0	15 959	41.4	961	17.9	345	18.2	24 906
1996	2 174	45.0	1 095	10.6	4 278	42.0	15 358	39.8	849	15.8	423	22.3	24 177
1997	2 054	43.0	1 047	10.2	4 148	41.0	13 967	36.1	783	14.5	356	18.7	22 355
1998	2 118	44.0	1 065	10.3	3 999	39.0	13 302	34.4	744	13.8	346	18.2	21 574
1999	1 770	37.0	990	9.6	3 912	39.0	12 168	31.0	651	12.9	316	16.6	19 807
Total	21 645		12 242		40 164		153 419		9 860		3 710		241 040

Explanations:
abs. = absolute number of bacteriologically confirmed cases of *M. tuberculosis* infection
rel. = relative number of bacteriologically confirmed cases of *M. tuberculosis* infection per 100 000 inhabitants

Table 3. *M. tuberculosis* infection in animals in six Central European countries

Country	Year	Cattle[1]	Swine[2]	Elephant[3]	Agouti[4]	Tapir[5]	Total
Croatia	–	0	0	0	0	0	0
Czech Republic	–	0	0	0	0	0	0
Hungary	–	0	0	0	0	0	0
Poland	1994	1	0	0	0	0	1
	1995	3	0	0	0	0	3
	1996	1	0	0	0	0	1
	1997	0	1	1[6]	1[6]	0	3
	1998	1	0	0	0	0	1
	1999	3	0	0	0	1[6]	4
Slovakia	1994	0	1	0	0	0	1
	1997	0	1	0	0	0	1
	1998	0	1	0	0	0	1
Slovenia	–	0	0	0	0	0	0
Total		9	4	1	1	1	16
%		56.1	25.0	6.2	6.2	6.2	100

Explanations:
[1]cattle (*Bos taurus*) from a small cattle farm with less than 9 cows; [2]domestic swine (*Sus scrofa* f. *domestica*); [3]african elephant (*Loxodonta africana*); [4]agouti (*Dasyprocta aguti*); [5]tapir (*Tapirus terrestris*); [6]zoological garden in Gdansk, Poland

from a different farm and in all cases these farms had less than 9 cows in a herd. The pigs also came from small farms in which feeding of pigs continued to a slaughter weight of 115 kg.

In **Slovakia** *M. tuberculosis* was isolated from three domestic pigs which came from three different farms with a size of 100 to 200 pigs fed to a slaughter weight of 115 kg.

The occurrence of *M. tuberculosis* in wild animals in the years 1990 to 1999

In Poland *M. tuberculosis* was isolated from three wild animals (Table 3): from one African elephant (*Loxodonta africana*), one agouti (*Dasyprocta aguti*) and one terrestrial tapir (*Tapirus terrestris*) in zoological garden in Gdansk in 1997 and 1999.

DISCUSSION

Even after the control of bovine tuberculosis in cattle and other species of domestic animals, tuberculous changes in the lymph nodes of pigs and cattle continued to be found during veterinary meat inspections in slaughterhouses (Pavlik *et al.*, 2002c). However, particularly in pigs alterations are often caused by other mycobacterial species, of which the most frequently represented are *M. avium* complex isolates (Pavlik *et al.*, 2000a, 2003). These and atypical mycobacteria have contaminated, for example, sawdust, drinking water or peat given as a food supplement (Pavlik *et al.*, 1999, 2000b). Apart from the *M. avium* complex isolates and atypical mycobacteria, *Rhodococcus equi* has also been isolated from the tuberculous changed lymph nodes of cattle and pigs (Dvorska *et al.*, 1999). On the basis of the results given in Table 2, one must, however, take into account the possible role of *M. tuberculosis* in any finding of tuberculous lesions in the lymph nodes of animals.

The infection of animals with *M. tuberculosis* also may complicate intravital diagnosis. During this study it was found that cattle naturally infected with *M. tuberculosis* reacted positively to skin test with bovine tuberculin (Andres, 1950; Hubrig, 1957; Pavlas and Rademachr, 1959; Krajsa, 1959; Pavlas *et al.*, 1964; Pavlas and Mezensky, 1982). These discoveries in the field were later confirmed also in experimentally infected cattle (Pavlas, 1965).

From the epizootiological point of view it is important to know the risks represented by the source of *M. tuberculosis* for herds of animals. In zoological garden in Gdansk (Poland), *M. tuberculosis* was isolated from three animals. These animals were kept in different parts of the zoological garden, from which it can be judged that the possibility of mutual transmission of infection was minimal. As humans most frequently have tuberculosis caused by *M. tuberculosis*, they may act as the primary source of infection for animals in zoological gardens (Thoen and Steele, 1995). Domestic and wild animals kept in captivity are most frequently put at risk sick attendants or visitors who feed animals with food often contaminated with their saliva and nasal discharges. In this way animals kept for example in zoological gardens, national parks and other establishments are put at risk of *M. tuberculosis* infection not only from attendants, but also from visitors.

Patients with tuberculosis of the urogenital tracts may excrete *M. tuberculosis* through urine. Therefore it is appropriate in zoological gardens to restrict direct or indirect contact with visitors, for example by locating exhibits behind a glass barrier. In agricultural enterprises testing for human tuberculosis should be required especially workers from countries with high prevalence of human tuberculosis (Ukraine, Belarus and others). Domestic pets, mainly dogs and cats are highly exposed to human tuberculosis because of their close contact with humans.

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