Mycobacterial infections in cattle in the Czech Republic during 1990–1999

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ABSTRACT: In the nineties, cattle stocks gradually decreased from 3 506 222 head of cattle in 1990 to 1 657 337 head in 1999. Skin testing of cattle was carried out annually using bovine tuberculin. Animals for sale were also simultaneously tested with avian tuberculin. In records from 1991 to 1999 a total of 14 611 393 bovine tuberculin skin tests and 611 405 simultaneous avian tuberculin skin tests are registered. A total of 1 457 (0.01%) animals reacted positively with bovine tuberculin and 1 790 (0.29%) with avian tuberculin. In the period monitored a total of 7 268 274 head of cattle were slaughtered and given veterinary hygienic examinations. Statistical data on the *post-mortem* detection of tuberculous lesions have been available for nine years since 1992 when tuberculous lesions were found in 1 186 (0.019%) out of 6 273 441 slaughtered animals. Mycobacteria were isolated from the organs of only 561 (17.5%) out of 3 202 culturally examined animals. *M. bovis* only was isolated from 48 (8.6%) animals originating from seven herds (two infected herds in 1991, 1992 and 1994 and one infected herd in 1995): four outbreaks were detected by annual skin testing, one outbreak by movement tuberculin skin testing and two outbreaks by the detection of tuberculous lesions at slaughter. *M. avium* complex strains of serotypes 1, 2 and 3 and of genotypes IS*901*+ and IS*1245*+ were isolated from 331 (59.0%) animals and strains of serotypes 4 to 6, 8 to 11 and 21 and of genotypes IS*901*- and IS*1245*+ were isolated from 132 (23.5%) animals. Potentially pathogenic bacteria of the *M. chelonae*, *M. terrae*, *M. phlei* and *M. fortuitum* species were isolated from 50 (8.9%) animals. Neither miliary nor generalised tuberculosis was found in any of the animals. Between 1996 and 1999, the proportion of cattle in which tuberculous lesions were recorded decreased.

Keywords: bovine tuberculosis; *Mycobacterium bovis*; *Mycobacterium avium* complex; IS*901*; IS*1245*; skin test

Bovine tuberculosis in cattle was controlled in the Czech Republic in 1968 (Polak, 1969; Kouba, 1988, 1999; Pavlas, 1999). In the post-elimination period, between 1969 and 1979, the incidence of bovine tuberculosis in cattle gradually decreased from 61 outbreaks in 1971 to 9 outbreaks in 1979. Only one outbreak was found in 1980, and between 1981 and 1989 only 9 outbreaks of bovine tuberculosis in cattle were detected (Chloupek, 1981; Pavlik *et al.*, 1998). Between 1990 and 1999, only 7 outbreaks were found, the last being recorded in 1995 (Pavlik *et al.*, 1998, 2001, 2002a,c). *Mycobacterium bovis* was not found in cattle in the Czech Republic between 1996 and 1998. In 1999 *M. bovis* was isolated from an individual of red deer (*Cervus elaphus*) which was kept on a farm (Pavlik *et al.*, 2002b).

Despite this improving situation, surveillance testing for tuberculosis using bovine tuberculin was carried out in the Czech Republic at least once yearly in cattle older than 24 months. During *post-mortem* inspection of cattle that had reacted to

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tuberculin and of other cattle, tuberculous lesions in lymph nodes were further examined for mycobacteria in a laboratory. Apart from the occasional detection of *M. bovis* strains, the main isolates were strains of the *M. avium* complex by the late 1980s (Pavlas, 1990, 1999). For example, between 1975 and 1979, out of 5 765 head of cattle, *M. bovis* was isolated from 312 animals, *M. avium* from 998 animals and atypical mycobacteria from 141 animals (Krucky, 1981). It was found that atypical mycobacteria from feed, water, bedding and in barns and the external environment were responsible for many of the non-specific reactions to tuberculin testing (Hejlicek *et al.,* 1982; Pavlas, 1990).

The occurrence of *M. bovis* in cattle and other animals in the Czech Republic between 1990 and 1999 was sporadic (Pavlik *et al.*, 1998, 2001, 2002b,c). Strains of the *M. avium* complex (Kovarik *et al.*, 1995), *Rhodococcus equi* (Dvorska *et al.*, 1999) and other species of bacteria (Docekal *et al.*, 1995) were commonly isolated from tuberculous lesions.

The aim of the present paper was to analyse the results of tuberculin testing and *post-mortem* diagnoses of bovine tuberculosis in cattle between 1990 and 1999.

MATERIAL AND METHODS

Sources of statistical data

Milk yields per cow and cattle stocks kept in the Czech Republic between 1990 and 1999 were obtained from official statistics of the Ministry of Agriculture of the Czech Republic (Table 1). The State Veterinary Administration of the Czech Republic (SVA CR) collated data on tuberculin testing and laboratory examinations each calendar year. Although tuberculin testing in cattle over 24 months old was carried out every year, data were available only from 1991 onwards. The results of *post-mortem* inspections in slaughterhouses

Table 1. Statistical data on cattle husbandry in the Czech Republic and bovine tuberculosis in cattle herds

Year[1]	No. of		Kg of milk per cow[4]	No. of outbreaks (herds)*		
	cattle[2]	cows[3]		large	small	total
1990	3 506 222	1 236 218	4 038	0	0	0
1991	3 359 976	1 195 429	4 031	2	0	2
1992	2 949 574	1 036 276	3 992	1	1	2
1993	2 511 737	932 454	4 093	0	0	0
1994	2 161 438	829 729	4 158	2	0	2
1995	2 029 827	768 236	4 453	0	1	1
1996	1 988 810	750 593	4 680	0	0	0
1997	1 865 902	702 301	4 849	0	0	0
1998	1 700 789	646 838	5 079	0	0	0
1999	1 657 337	642 026	5 478	0	0	0
Total				5	2	7

Explanations:

[1]cattle stocks recorded in 1990 to 1991 on January 1st and in 1992 to 1999 on March 1st

[2]total number of cattle

[3]number of cows out of the total number of cattle

[4]annual production of milk for the standardised lactation of one cow

*published previously (Pavlik *et al.*, 1998, 2002c): **large herd**: catle herd with ≥ 10 cows, **small herd**: cattle herd with < 9 cows

Table 2. Skin testing of cattle with bovine and avian tuberculins in the Czech Republic between 1990 and 1999

Year	Mammalian/Bovine tuberculin			Avian tuberculin		
	number of animals			number of animals		
	tested	positive	%	tested	positive	%
1990	nr	nr	nr	nr	nr	nr
1991	3 831 518	0	0.00	60 986	75	0.12
1992	2 238 842	321	0.01	78 642	434	0.55
1993	1 865 469	301	0.02	115 165	393	0.34
1994	1 634 547	124	0.01	132 909	278	1.21
1995	1 539 145	340	0.02	90 260	293	0.32
1996	1 386 218	124	0.01	66 159	247	0.37
1997	934 748	61	0.01	27 916	17	0.06
1998	468 977	109	0.02	21 572	19	0.09
1999	711 929	77	0.01	17 796	34	0.19
Total	14 611 393	1 457	0.01	611 405	1 790	0.29

Explanations:
nr = done, but not registered

were available for the same period except for 1991 (Tables 2 and 3).

Between 1990 and 1995, the data from the veterinary hygiene service were collated for the whole calendar year. In 1996, as a part of measures leading towards the Czech Republic becoming a member of the European Union, the hygiene year began in January and ended in October of the same year (only 10 months were evaluated). In the following period, the hygienic year always began in November of the previous year and finished in October of the following year (12 months evaluated).

Tuberculin testing of cattle until May 1993

Tuberculin testing was carried out once yearly on all animals over 24 months old using bovine tuberculin. Until 31 May 1993, 0.2 ml of purified mammal tuberculin (25 000 TU/ml, Bioveta, Ivanovice na Hane, Czech Republic) manufactured from strains of *M. tuberculosis* and *M. bovis* No. AN 5 and 0.2 l of purified avian tuberculin for simultaneous testing (8 000 TU/ml, Bioveta, Ivanovice na Hane, Czech Republic) manufactured from *M. avium* subsp. *avium* strain No. D 4 ER were used.

For single intradermal tuberculin testing using mammal tuberculin, each reaction was classified by a reaction number (RN); RN being a difference in skin thickness in mm before and 72 hours after intradermal tuberculin testing. RNs less than 1.5 mm in herds infected with bovine tuberculosis and less than 2.5 mm in herds free of bovine tuberculosis were classified as negative. RN 3.6 mm or more, accompanied by soreness and enlargement of the relevant pre-scapular lymph node, was considered positive. All other reactions, corresponding neither to negative nor to positive result, were considered doubtful. Animals with positive or doubtful reactions were re-tested by comparative intradermal tuberculin testing carried out after 42 days at the earliest. A key was used for evaluating the reaction (Table 4; Pavlas, 1990).

Tuberculin testing of cattle since June 1993

After June 1, 1993, as a part of the association of the Czech Republic with the European Union,

Table 3. Results of examinations of slaughtered cattle for tuberculosis between 1990 and 1999 and cultivation examination for mycobacteria

Year	Number of animals				Isolated mycobacteria							
	slaughtered*	with TB	%	cultured**	total	%	*M. bovis*	*M. avium* complex				other spp.
								total	*M. a.*	*M. i.*	*a./i.*	
1990	1 041 782	138	0.013	570	68	11.9	0	64	54	10	5.4	4
1991	994 833	nk	nk	534	58	10.9	11[1]	47	40	7	5.7	0
1992	944 909	100	0.011	365	78	21.4	2[2]	66	42	24	1.8	10
1993	812 570	126	0.016	329	72	21.9	0	61	37	24	1.5	11
1994	700 927	163	0.023	853	102	12.0	6[3]	84	48	36	1.3	12
1995	609 172	157	0.026	200	81	40.5	29[4]	42	30	12	2.5	10
1996	580 064	183	0.032	143	61	42.7	0	59	46	13	3.5	2
1997	586 439	107	0.018	76	21	27.6	0	20	15	5	3.0	1
1998	528 613	109	0.021	69	9	13.0	0	9	9	0	0	0
1999	468 965	103	0.022	63	11	15.6	0	11	10	1	10.0	0
Total[5]	7 268 274	nk	nk	3 202	561	17.5	48	463	331	132	2.5	50
Total[6]	6 273 441	1 186	0.019									
%				100			1.5	14.5	10.3	4.1		1.6
%					100		8.6	82.5	59.0	23.5		8.9

Explanations:

TB = tuberculosis lesions in lymph nodes or parenchymatous organs (the result of the examination of the whole bodies of slaughtered animals, adjudged consumable with limitation or condemned)

M. a. = *M. avium* (virulent strains for poultry causing generalised avian tuberculosis, or strains of serotypes 1, 2 and 3 or genotypes IS*901*+ and IS*1245*+

M. i. = *M. intracellulare* (strains partially virulent for poultry causing tuberculous lesions only at the place of inoculation, or strains of serotypes 4 to 6, 8 to 11 and 21 or genotypes IS*901*– and IS*1245*+)

a./i. = ratio of *M. avium* to *M. intracellulare* strains

nk = exact data not known

Other spp. = *M. terrae*, *M. chelonae*, *M. phlei*, *M. triviale* and others

*in the hygienic year data was gathered from 1990 to 1995 from January to December, in 1996 from January to October and from 1997 to 1999 always from November of the previous year to October of the year evaluated

**cultivation examinations for mycobacteria were always evaluated from January to December of the relevant calendar year

[1]all 11 animals came from two infected farms at District Veterinary Administration (DVA) Prague-East (detected by annual skin testing)

[2]*M. bovis* isolated from one cow from milk and tuberculosis-changed lung tissue at DVA Ceske Budejovice (detected by annual skin testing) and from one cow from tuberculosis-changed bronchial lymph nodes at DVA Prachatice (detected by annual skin testing)

[3]*M. bovis* isolated from one bull in feed at DVA Uherske Hradiste (detection of tuberculous lesions in lung lymph nodes at slaughter) and from 5 bulls at DVA Znojmo (detected by movement tuberculin skin testing)

Table 4. Key for assessing tuberculin testing in herds where the infection with *M. bovis* was not identified (amended according to the paper of Pavlas, 1990)

Assessment of RN for skin test		Ratio of RN for both tuberculins	Result of tuberculin testing/infection	
mammal	avian		*M. bovis*	*M. avium**
≤ 2.5	≤ 2.5		–	–
	2.6–3.5		–	–
	≥ 3.6		–	–
2.6–3.5	≤ 2.5		+–	–
	2.6–3.5		+–	+–
	≥ 3.6		–	+
3.6–5.9	≤ 2.5		+	–
		RN for mammal tuberculin is equal or predominates by <3.0	+	–
	≥ 2.6	RN for mammal tuberculin predominates by > 3.0	+	–
		RN for avian tuberculin predominates	–	+
≥ 6.0		RN for avian tuberculin predominates by > 3.0	–	+
		RN for avian tuberculin predominates by < 3.0	+–	+–
		RN for mammal tuberculin predominates	+	–

Explanations:

RN = reaction number (ratio of skin thickness before and after intradermal tuberculin testing after 72 hours in mm)
*sensibilisation through *M. avium* complex strains or other atypical mycobacteria

the bovine tuberculin used was manufactured from *M. bovis* (strain No. AN 5, labelled BOVITUBAL, 14 000 TU/ml) and the avian tuberculin was manufactured from *M. avium* subsp. *avium* strain (D 4 ER, labelled AVITUBAL for comparative intradermal test, 14 000 TU/ml).

Under **single intradermal tuberculin testing** with bovine tuberculin, RN higher than or equal to 4.0 mm was considered as a positive reaction, RN between 2.0 and 3.9 mm as doubtful and reaction number lower than 1.9 mm as negative. Under **comparative intradermal tuberculin testing**, the results began to be interpreted as follows:

Positive: reaction to bovine tuberculin exceeded by more than 4 mm the reaction to avian tuberculin and clinical symptoms did not appear at the point of application of the bovine tuberculin (diffuse or widespread swelling, exudation, necrosis, soreness or infectious reaction of the relevant lymph vessels or lymph nodes).

Doubtful: reaction to bovine tuberculin was positive or doubtful and the reaction was not more than 4 mm greater than the reaction to avian tuberculin and none of the above-mentioned clinical symptoms appeared.

Explanations to Table 3 to be continued:

[4]all 29 animals came from one herd of cattle at DVA Zdar nad Sazavou (detection of tuberculous lesions in lung's lymph nodes and tisuue at slaughter in one cow), in which infection was caused by bovine tuberculosis also found in five domestic pigs kept together with them (Pavlik *et al.*, 2002a)

[5]total of animals slaughtered over the whole period monitored

[6]total of animals slaughtered excluding 1991, when the results of veterinary hygienic examination for tuberculosis were not available

Negative: reaction to bovine tuberculin was positive, doubtful or negative, but the reaction was the same or less than to avian tuberculin and in both cases none of the above-mentioned clinical symptoms were found.

Post-mortem examination of cattle at slaughter

Cattle, including calves, may be slaughtered only in slaughterhouses in the Czech Republic. To protect the human population against infection with *M. bovis*, all lesions resembling tuberculosis found in slaughtered cattle are examined in specialised laboratories. The veterinary hygienic assessment of meat and organs from slaughtered cattle was conducted according to SVA CR Directive No. 1 of 21 September 1989, Article 4, Appendix No. 1 "Principles for Deciding about the Meat and Organs of Slaughter Animals", which required the following lymph nodes were assessed by inspection and incision immediately after slaughter: *Lymphonodi* (*Lnn.*) *mandibulares*, *Lnn. tracheobronchales sinistri, mediales* and *dextri*, *Lnn. tracheobronchales dextri*, *Lnn. mediastinales*, *Lnn. hepatici*, *Lnn. gastrici*, *Lnn. jejunales* and *Lnn. inguinales superficiales*.

If pathological lesions were found in any of the above-mentioned organs during the foregoing investigation, a more detailed veterinary examination was carried out. Incisions were made in suspect areas and lymph nodes in their vicinity and regional lymph nodes. Caseated or even calcified tubercles of different size and shape (most frequently from the size of a poppy seed to that of a pin-head) were considered as tuberculous changes. All adjacent organs or parts of the animal close to the tuberculosis-changed lymph nodes were collected for laboratory examination and the whole carcass was classed as a conditionally consumable product (i.e. destined for heat treated products).

Culture for mycobacteria

Tissues for laboratory examination were chilled to 4°C frozen to –20°C and delivered for examination within 2 to 3 weeks from slaughter. Tissue culture for mycobacteria was conducted in three laboratories: Reference Laboratory for the Diagnosis of Tuberculosis in Animals at the State Veterinary Diagnostic Institute in Prague, Mycobacteriological Laboratory at the State Veterinary Diagnostic Institute in Brno, and SVS CR Methodological and Consultation Centre for Tuberculosis, Paratuberculosis and Mycobacterial Infections in Animals and Office International des Epizooties (OIE), Reference Laboratory for Paratuberculosis at Veterinary Research Institute in Brno. The method for the laboratory examination for mycobacteria was described earlier (Fischer *et al.*, 2000).

Identification of isolated mycobacterial strains

The isolated mycobacterial strains were determined using biochemical methods (Wayne and Kubica, 1986) and since 1993 using Accu Probe (Accu-Probe Inc., San Diego, California) for the identification of *M. tuberculosis* complex strains. *M. avium* complex strains were identified through serotyping (Wolinsky and Schaefer, 1973; Süssland and Hrdinova, 1976), biological experiment on poultry after intramuscular infection (Pavlas and Patlokova, 1977) or, since 1996, using the PCR method for detecting IS*901* (Kunze *et al.*, 1992; Pavlik *et al.*, 2000a).

RESULTS

From 1991 to 1999, the reported stocks of cattle and cows kept in the Czech Republic decreased. In 1999 only 47.3% head of cattle were slaughtered in comparison with the number of animals slaughtered in 1990. The steep fall in the number of slaughtered animals was compensated by an increase in milk production efficiency that has shown an unequivocally upward trend since 1992 (Table 1).

Tuberculin testing of cattle

In the years between 1991 and 1999, a total of 14 611 393 single tuberculin tests were carried out using bovine tuberculin. A positive reaction was found in 1 457 (0.01%) animals that were either slaughtered or subjected to comparative tuberculin testing. Comparative tuberculin testing was also obligatorily carried out in all animals transferred out of their herd of origin. Comparative tuberculin testing was carried out in 611 405 animals and positive reactions were found in 1 790 (0.29%) animals (Table 2).

Outbreaks of bovine tuberculosis in cattle

Only seven outbreaks of bovine tuberculosis in cattle were found in the years 1991, 1992, 1994 and 1995. Detection of bovine tuberculosis occurred mainly in larger herds with 10 or more cows (Table 1).

Tuberculous lesions in cattle and their laboratory examination

Over the period, 7 268 274 head of cattle were slaughtered and results of *post-mortem* inspection were available for 6 273 441 head of cattle over nine years (all years except 1991). Overall tuberculous lesions were found in 1 186 (0.019%) head (range 0.013 to 0.032% per year). In the year 1990, and from 1992 to 1995, more animals were further examined in laboratories than the number of slaughtered animals found to have tuberculous lesions (Table 3) because autopsy material from animals that reacted positively during tuberculin testing was also sent for culture.

Tissues from 3 202 head were examined for the presence of mycobacteria: they were identified in 561 (17.5%) animals in total. The proportion of cattle from which mycobacteria were isolated ranged from 10.9% to 42.7% annually (Table 3).

Isolation of *M. bovis*. *M. bovis* was isolated in four years only – 1991, 1992, 1994 and 1995 – from 48 slaughtered animals coming from 7 officially declared outbreaks of bovine tuberculosis: four outbreaks were detected by annual skin testing, one outbreak by movement tuberculin skin testing and two outbreaks by the detection of tuberculous lesions at slaughter (Table 3). Most of the infected animals (29) came from the last outbreak of bovine tuberculosis in cattle in the Czech Republic described earlier (Table 3; Pavlik *et al.*, 2001, 2002a). In all cases, *M. bovis* was isolated from the lung tissue or bronchial lymph nodes with tuberculous lesions. In 1992 *M. bovis* was also isolated from the milk of one cow with tuberculous lesions in the bronchial lymph nodes (Table 3).

Isolation of *M. avium* complex strains. From a total of 561 strains isolated, 463 (82.5%) belonged to the *M. avium* complex (Table 3). On the basis of the results of serotyping, experimental *intramuscular* inoculations of pullets and examinations using the IS*901* and IS*1245* PCR method, it was possible to divide strains into strains of serotype 1 to 3, IS*901*+ and IS*1245*+, virulent for birds, of which there were 331 (71.5%) and strains of serotypes 4 to 28, IS*901*– and IS*1245*+, not virulent for birds, of which there were only 132 (28.5%). Overall 2.5 times more strains were virulent for birds than not virulent (Table 3).

Isolation of strains of other mycobacterial species. From a total of 561 strains isolated, only 50 (9.9%) were identified as species other than strains of the *M. bovis* species or the *M. avium* complex. These strains most frequently belonged to the conditionally pathogenic mycobacterial species *M. chelonae*, *M. terrae*, *M. phlei* and *M. fortuitum* (Table 3).

DISCUSSION

A decrease in the number of samples sent for laboratory examinations corresponded with a reduction in the number of cattle head slaughtered over the period (Table 3). As a result of the eradication of bovine tuberculosis in 1968, a reorganisation of the bacteriological diagnosis of *M. bovis* took place in the early 1970s (Krucky, 1973). Since then, all authorised veterinary laboratories have been involved in the histological diagnosis of tuberculous lesions. Tissue culture is conducted in three specialised mycobacterial laboratories only. Until 1995, biological material was always culturally examined from more animals than those found with tuberculous findings (Table 3). It was because standard tissues from animals that reacted positively during tuberculin testing were also sent for culture to identify the causes of the reactions. Since 1996, however, the number of cultured samples was lower than the number of animals in which tuberculous lesions were found (Table 3). This situation may be considered unsatisfactory at a time when the eradication of tuberculosis is nearing completion.

It is evident from the results in Table 1 that the occurrence of only seven outbreaks of cattle in the period was very low. These herds were depopulated within a few months of being identified. However, if a new outbreak of bovine tuberculosis occurs, inadequate follow-up examinations may delay identification of tuberculous lesions and allow *M. bovis* to spread in the absence of disease control measures. This occurred in the last outbreak of bovine tuberculosis in the Czech Republic in 1995 (Pavlik *et al.*, 2001, 2002a). It is therefore still necessary to recommend thorough health examinations before transfers of animals (tuberculin testing) and

further laboratory examination of all tuberculous lesions found in slaughtered cattle. Tissue culture is also necessary to rule out *M. bovis* infection as other bacteria may also be involved causing tuberculous lesions (Docekal *et al.*, 1995; Dvorska *et al.*, 1999).

The number of animals with tuberculous lesions did not exceed 0.04% in any of the years documented (Table 3). In comparison with the frequency of tuberculous lesions in pigs, this occurrence was lower 0.32% of slaughtered pigs in 1989 to 1999 (Pavlik *et al.*, 2000b). The cause of the more frequent findings of tuberculous lesions in pigs than in cattle was the use of potentially risky bedding (sawdust and wood-shavings) or supplementary feeding of piglets with peat that were often contaminated with atypical mycobacteria, especially strains of the *M. avium* complex (Pavlik *et al.*, 1999).

In the period we monitored, other species of mycobacteria besides *M. bovis* were often involved in skin reactions to bovine tuberculin and in the occurrence of tuberculous lesions. From an epidemiological point of view, the sources of mycobacterial infections may be divided into the groups given below:

1. *M. bovis* was mainly detected in old cows in which tuberculin test was negative (anergic animals) from 1991 and 1995 (Plhal, 1992; Pavlik *et al.*, 1998, 2001, 2002a).
2. The sources of strains of the *M. avium* complex (serotypes 1 to 3, IS*901*+ and IS*1245*+) for cattle were probably infected wild birds living in the wild (Hejlicek and Treml, 1993; Pavlik *et al.*, 2000a). The infection could, however, have also been brought into herds by infected small rodents which migrate for food to the barns of cattle farms in the autumn and winter months. Infection with these strains has been identified in these rodents (Fischer *et al.*, 2000). Outside the host organism, these strains occur in the external environment very rarely (Horvathova *et al.*, 1997).
3. The source of other serotypes (4 to 28) of the *M. avium* complex and atypical mycobacteria (e.g. *M. terrae*, *M. chelonae*, *M. phlei* and *M. triviale*) was most probably the external environment where these strains most frequently occur (Horvathova *et al.*, 1997). Strains of these serotypes of the *M. avium* complex were also isolated from cold-blooded animals, invertebrates and small vertebrates that may also be their source or vectors under certain circumstances (Matlova *et al.*, 1998; Fischer *et al.*, 2000, 2001).

CONCLUSIONS

It is necessary to know the causes of tuberculous lesions in cattle to facilitate the timely introduction of disease control measures to prevent the spread of infection. In this way, economic losses to breeders, including limitation of the risk of possible mycobacterial infection in human beings, should be reduced. For this reason, it is always necessary to observe the following principles:

1. Continue the regular tuberculin testing with bovine tuberculin of cattle over 24 months old and all animals before transfer out of the herd.
2. In slaughterhouses, conduct a thorough veterinary hygienic examination of all animals slaughtered.
3. Do thorough cultivation examinations of biological material from cattle for the presence of mycobacteria (above all, tuberculosis-changed lymph nodes and tissue of parenchymatous organs and lymph nodes of positive reagents during tuberculin testing).
4. Laboratory examine tuberculosis-changed tissues found during veterinary hygienic examinations from other domestic ruminants and pigs and from wild animals.
5. Introduce laboratory methods for the direct detection of *M. bovis* in tuberculosis-changed tissues to speed up diagnosis.

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