

A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia

J.E. SHITAYE^{1,2}, B. GETAHUN³, T. ALEMAYEHU⁴, M. SKORIC², F. TREML², P. FICTUM², V. VRBAS¹, I. PAVLIK¹

¹Veterinary Research Institute, Brno, Czech Republic

²University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

³Addis Ababa Abattoirs Enterprise, Addis Ababa, Ethiopia

⁴Shola Veterinary Clinic and Laboratory, Addis Ababa, Ethiopia

ABSTRACT: *Post mortem* surveillances, for the detection of tuberculous lesions in particular depend on the work load time and the diligence of the inspector conducting the examination. The first aim of the study was to determine the trend of occurrence of tuberculous lesions in two abattoirs in Addis Ababa and Debre-Zeit (Ethiopia). The second aim of the study was to determine prevalence of the tuberculin skin test results in 10 dairy farm areas in Addis Ababa. The third aim was to detect tuberculous lesions and causal agents from tissue samples of the respiratory tract and mesenteric lymph nodes of the slaughtered cattle. The ten year (1996–2005) retrospective analysis of the meat inspection of 2 455 289 slaughtered animals showed that 707 (0.028%) were found with tuberculous lesions in parenchymatous organs of which were 699 (0.052%) of 1 336 266 cattle, 4 (0.001%) of 534 436 sheep, 3 (0.001%) of 573 767 goats and 1 (0.009%) of 10 820 pigs. The tuberculous lesions found in cattle were statistically highly significant ($P < 0.01$) than in other animals. The bovine tuberculin skin tests were conducted in Addis Ababa in 10 farm areas in 85 dairy farms having 2 098 cattle. Positive reactions were obtained from 9 farm areas in 41 (48%) herds which included 392 (19%) of the animals. In a current study, tuberculous lesions were found in 34 (3.5%) animals by the meat inspection surveillance of 984 cattle. Histopathologically, granulomatous inflammation was evident in 3 (8.8%) animals with tuberculous lesions. A highly sensitive PCR (IS6110) was positive in 4 of 34 (11.8%) animals with tuberculous lesions and in 1 (2.9%) of animal without lesions. The analyzed data and these study findings indicated that tuberculosis in cattle is an existing problem in Ethiopia which needs to be solved.

Keywords: cattle; *M. bovis*; economic losses; veterinary meat inspection; zoonosis; Africa; food safety

Tuberculosis is a chronic bacterial disease in animals and humans characterized by the progressive development of specific granulomatous lesions of tubercles in affected tissues. The disease affects all age groups of susceptible hosts and is accountable for more deaths through out the world than any other bacterial disease ever today (Omer et

al., 1995). Tuberculosis in cattle and other domestic animals is above all caused by two members of *Mycobacterium tuberculosis* complex (MTC): *M. bovis* and *M. caprae* (Pavlik et al., 2002; Prodinger et al., 2002; Erler et al., 2004). However, occasional occurrence of tuberculosis due to *M. tuberculosis* species with concurrent tuberculous lesions has

Supported by the Ministry of Agriculture of the Czech Republic (Grant No. MZE 0002716201).

been reported in pigs (Lesslie and Brin, 1970; Popluhar et al., 1974; Flesja et al., 1978), in cattle (Popluhar et al., 1974; Schliesser, 1976; Thoen et al., 1981; Pavlik et al., 2005), in dogs (Pavlik et al., 2003) and other animals (Pavlik, 2006).

The global prevalence of human tuberculosis due to *M. bovis* has been estimated at 3.1% of all human tuberculosis cases, accounting for 2.1% and 9.4% of pulmonary and extra pulmonary TB cases respectively (Cosivi et al., 1998). Drinking raw milk is a primary route of *M. bovis* infection in humans; hence the occurrence of human tuberculosis is most commonly extra pulmonary form, particularly in the cervical lymphadenitis form. Recently, Kidane et al. (2002) indicated that *M. bovis* along with other *MTC* species were found to be a cause for tuberculous lymphadenitis in humans in Ethiopia. According to Enarson (2006) literature review more than 50% of *M. bovis* infection in humans in Europe, USA, Canada, Argentina, Australia and New Zealand has pulmonary localization. This proportion may however, change in other regions of the world, especially in Africa. In contrast to this report, cases of pulmonary tuberculosis due to *M. bovis* were also reported (O'Reilly and Daborn, 1995; Kazwala et al., 2001).

Currently, due to the upsurge of *HIV/AIDS* infection, the epidemiology of tuberculosis has been greatly affected as many *HIV*-infected individuals are co-infected with tuberculosis, the incidence of the disease may rise in coming years (Zumla et al., 1999; Ayele et al., 2004). The occurrence of *M. bovis* in humans, against the background of the soaring *HIV/AIDS* incidence particularly in eastern and southern Africa, implies that the risk of spillover of zoonotic tuberculosis to rural communities is rapidly increasing (Zinsstag et al., 2006). Moreover, Regassa (2005) reported that a higher prevalence of tuberculosis in cattle owned by tuberculous patients was found than in cattle owned by non tuberculous owners, which suggests the significant role of *M. bovis* in the incidences of human tuberculosis in Ethiopia. Thus, the correlation between the prevalence of *M. bovis* infection in humans and that of local cattle populations highlights the potential threat of this disease for humans (Daborn et al., 1996).

In developing countries, animal tuberculosis is still prevalent and is responsible for significant economic loss in animal production and an increase in human health problems and even deaths (Neill et al., 1994; Cosivi et al., 1998; Pollock and Neill,

2002). According to literature data, approximately 85% of the cattle and 82% of the human population of Africa live in areas where animal tuberculosis is either partly controlled or uncontrolled. In contrast however, only a few African countries have applied disease control measures as part of "test and slaughter" strategy and consider the disease as notifiable (Cosivi et al., 1998). Despite this, in most developing countries pasteurization is not well practiced and therefore, 10% to 15% of human tuberculosis is considered to be caused by *M. bovis* (Ashford et al., 2001).

In Ethiopia, the endemic nature of tuberculosis in cattle has long been reported (Hailemariam, 1975) and is one of the countries, where tuberculosis is wide spread in both human and cattle populations (Ameni, 1996; Kiros, 1998). However, due to the traditional extensive grazing system, which causes difficulties in the process of study, inadequate animal health infrastructures and less attention given to *M. bovis* infection, the actual prevalence of the disease is yet unknown (Abdo, 1993). Despite this, the economic impacts and zoonotic importance of the bovine tuberculosis infection are neither well studied nor documented. The very few studies indicated the prevalence of bovine tuberculosis ranges from 3.4% in small holder production system to 50% in intensive dairy productions (Ameni, 1996; Kiros, 1998).

Detection of bovine tuberculosis in cattle and other susceptible animal species is often made on history, clinical and necropsy findings, tuberculin skin tests and abattoir meat inspections. Definitive diagnosis is made on culture with morphological appearance and biochemical tests (Kent and Kubica, 1985) and molecular biological techniques like PCR (Boddinghaus et al., 1990; Cousins et al., 1991). In Ethiopia, however, due to technical and financial limitations, the new laboratory procedures have not yet being introduced as common diagnostic measures.

In view of increasing zoonotic tuberculosis in eastern and southern Africa (Zinsstag et al., 2006) this study was aimed to determine a trend in the occurrence of tuberculous lesions in two abattoirs in Addis Ababa and Debre-Zeit (Ethiopia) during the past ten years; to determine prevalence of the bovine tuberculosis based on tuberculin skin test results in 10 dairy farm areas in Addis Ababa and to detect tuberculous lesions and causal agents from tissue samples of the respiratory tract and mesenteric lymph nodes (ln) of slaughtered cattle in city abattoir of Addis Ababa.

MATERIAL AND METHODS

Part 1. Abattoir meat inspection analysis of tuberculous lesions

Data was collected from Addis Ababa and Debre-Zeit abattoirs from 1996 to 2005 (according to the Ethiopian budget year which starts on July 1st and finished on June 30th) and analyzed. The routine abattoir inspection for any infection including bovine tuberculosis applies the method developed by Meat Inspection and Quarantine Division of the Ministry of Agriculture, Ethiopia (Hailemariam, 1975). During observation of tuberculous lesions in various parts of the carcass: lung, intestine, liver, and corresponding ln, the whole carcass was condemned. In the case of localized lesions, partial condemnation of organs is undertaken. During this period, 2 455 289 domestic animals were slaughtered and inspected: 1 336 266 cattle, 534 436 sheep, 573 767 goats, and 10 820 pigs (Table 1).

Part 2. Analysis of tuberculin skin test results

Two years of bovine tuberculin skin test data (2004–2005) obtained from “Shola Veterinary Clinic and Laboratory” Addis Ababa (Ethiopia) was orga-

nized and statistically analyzed to determine the prevalence of bovine tuberculosis. To determine the sample size the formula as described by Thrusfield (1995) and simple random sampling was used to select the study farms. The comparative intradermal skin test was conducted on 2 098 cattle with the age of greater than six months in 85 different dairy farms originating from 10 farm areas in Addis Ababa. A skin test with a bovine tuberculin (50 000 TU produced from Strain Bovituber, Rhone Merieux GMBH, Germany) was used. For inoculation of tuberculin, the middle of the neck was shaved and the thickness measured with a 0.01 mm graduated caliper; then 0.1 ml of bovine PPD was injected intradermally. The injection sites were examined for swelling and thickness after 72 hours. The interpretation of the results was done according to (O.I.E., 2000).

Part 3. Abattoir meat inspection

Examination of animals for the detection of tuberculous cattle was undertaken in the Addis Ababa abattoir on 984 slaughtered bulls and culled cows (961 animals of zebu breed and 23 exotic breed cows and calves), out of which 9 were reactors for tuberculin skin test from Holleta genetic improvement center) from July to August 2005. During the routine meat inspection, observation of pathological

Table 1. Detection of tuberculous lesions in Addis Ababa and Debre-Zeit abattoirs (Ethiopia) in slaughtered animals

Year	Slaughtered animals			Cattle			Sheep			Goats			Pigs		
	No.	TB	%	No.	TB	%	No.	TB	%	No.	TB	%	No.	TB	%
1996	147 765	32	0.021	109 407	32	0.029	30 357	0	0	6 072	0	0	1 929	0	0
1997	148 215	36	0.024	112 667	36	0.032	29 855	0	0	4 115	0	0	1 578	0	0
1998	182 044	45	0.025	137 914	45	0.033	39 381	0	0	3 376	0	0	1 373	0	0
1999	277 012	50	0.018	106 878	50	0.047	67 711	0	0	101 824	0	0	599	0	0
2000	207 228	56	0.027	117 626	55	0.046	49 346	0	0	39 321	1	0.002	935	0	0
2001	190 922	37	0.019	138 044	37	0.026	39 120	0	0	12 974	0	0	784	0	0
2002	192 806	62	0.032	150 161	62	0.041	36 093	0	0	5 666	0	0	886	0	0
2003	267 802	106	0.039	150 977	106	0.070	47 213	0	0	68 762	0	0	850	0	0
2004	416 916	125	0.029	157 587	124	0.078	103 220	0	0	155 184	1	0.001	925	0	0
2005	424 579	158	0.037	155 005	152	0.098	92 140	4	0.004	176 473	1	0.001	961	1	0.104
Total	2 455 289	707	0.028	1 336 266	699	0.052	534 436	4	0.001	573 767	3	0.001	10 820	1	0.009

TB – number of animals with tuberculous lesions

lesions in the pleural cavity, lung, liver and intestine was marked. Based on these gross lesion findings a total of 69 tissue samples; 48 lung (bronchial and mediastinal ln), 15 mesenteric ln, 6 parenchymatous organ tissues (4 lung and 2 liver samples) were collected from 69 slaughtered animals.

Histopathological examination

After collection of samples, fat and other tissues were trimmed from ln and organs for histopathological examinations. Samples were then preserved in 10% buffered neutral formalin in a screwed glass container, closed tightly and kept under room temperature until examination. After transportation of samples to the laboratory of Institute of Pathological Morphology (University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic), all formalin fixed samples were dehydrated, embedded in paraffin wax and sectioned on microtome at a thickness of 4 µm. Histological samples were stained by hematoxylin and eosin (HE). Finding of granulomatous inflammation was taken as positive and other findings such as pyogranulomas and abscesses that can imitate macroscopically tuberculous nodules were taken as suspected results. The presence of Acid-fast bacteria (AFB) was examined by Ziehl-Neelsen (ZN) staining. A positive control for ZN staining was used.

Molecular biological test

All collected tissue samples fixed in 10% formalin were examined by PCR examination for identification of mycobacterial agents. The PCR mixtures were prepared from HotStar Taq™ Master Mix (Qiagen, Germany). Amplification with various sets of primers was used for the detection of specific DNA sequences. The IS6110 template specific for *MTC* members was amplified by the one-tube nested PCR system according to Cave et al. (1991) and Bartos et al. (2006).

Statistical evaluation of results

The *chi*² test (Stat Plus) was applied for statistical evaluation of results (Matouskova et al., 1992).

RESULTS

Part 1. Abattoir meat inspection analysis of tuberculous lesions

The retrospective meat inspection analysis (ten years) showed that among a total 2 455 289 slaughtered animals, 707 (0.028%) were found with tuberculous lesions: 699 (0.052%) of 1 336 266 cattle, 4 (0.001%) of 534 436 sheep, 3 (0.001%) of

Table 2. Bovine tuberculin skin test results in Addis Ababa in different dairy farm areas

Farm area	No. of tested farms		Tested animals						
	total	positive	total No.	reactors		positive		suspected	
				No.	%	No.	%	No.	%
Addis Ketema	5	2	107	45	42.1	44	44.1	1	0.9
Akaki-Kality	16	4	474	92	19.4	75	15.8	17	3.6
Arada	2	0	41	0	0	0	0	0	0
Bole	14	10	322	111	34.5	93	28.9	18	5.6
Gulele	8	3	242	26	10.7	26	10.7	0	0
Kirkos	4	2	50	4	8.0	4	8.0	0	0
Kolfe-Keranyo	12	7	358	71	19.8	66	18.4	5	1.4
Lideta	2	2	36	5	13.9	5	13.9	0	0
Nifas-silk	4	4	78	23	29.5	22	28.2	1	0.1
Yeka	18	7	390	66	16.9	57	14.6	9	2.3
Total	85	41	2 098	443	21.1	392	18.7	51	2.4
%	100	48.2							

573 767 goats, and 1 (0.009%) of 10 820 pigs (Table 1). Firm creamy whitish nodular focal lesions have been predominantly (63.9%) observed in the lung cavity and associated ln and very rarely in mesenteric ln and liver organs.

Frequencies of tuberculous lesions in sheep and goats were significantly lower ($P < 0.01$) than in pigs and cattle (Table 1) and tuberculous lesions in these species were commonly observed in the digestive tracts. The highest detection rates of tuberculous lesions were unequivocally recorded in cattle, particularly in 2004 and 2005; in comparison with the period between 1996 and 2002 with significant ($P < 0.01$) differences. The third highest detection rate of tuberculous lesions was recorded in 2003. All the values recorded in this year were significantly higher ($P < 0.05$) than in the period between 1996 and 2002.

Part 2. Analysis of tuberculin skin test results

The tuberculin skin test conducted in Addis Ababa dairy farms indicated that 41 (48.2%) of 85 herds were found as positive farms in 9 of 10 farm areas. From 2 078 tested animals 443 (21.1%) reacted to intradermal test, out of which 392 (18.7%) were positive and 51 (2.4%) were with suspected results (Table 2). The highest percentage of positive results in tested animals was recorded in the farm area of Addis Ketema (42.1%) where as the lowest percentage of positive results was found in the Gulele (10.7%) farm area. No positive cases were noted in the farm area of Arada (Table 2). The range of prevalence within herds was found to be 8.0% to 41.1% and the herd prevalence rate was significantly ($P < 0.01$) higher than individual prevalence rates.

Table 3. Examination of tissue samples from 69 cattle slaughtered in Addis Ababa abattoir

Examined samples	Total No.	Pathological lesions			Hematoxylin-Eosin staining ²				Ziehl-Neelsen		IS6110 PCR ³		
		stages ¹	No.	%	±	%	+	%	+	%	No.	%	
Lung ln ⁴	48	0	8	16.6	0	0	0	0	0	0	0	0	0
		1	15	31.3	15	31.3	0	0	0	0	0	0	0
		2	11	22.9	20	41.6	0	0	0	0	3	6.3	
		3	14	29.2	0	0	2	4.2	0	0	0	0	
Mesenteric ln ⁴	15	0	9	60.0	0	0	0	0	0	0	1	6.7	
		1	1	6.7	3	20.0	0	0	0	0	0	0	
		2	3	20.0	1	6.7	0	0	0	0	0	0	
		3	2	13.3	0	0	1	6.7	0	0	1	6.7	
Lungs and liver	6	0	2 ⁵	33.3	0	0	0	0	0	0	0	0	
		1	0	0	2	33.3	0	0	0	0	0	0	
		2	4 ⁶	66.7	2	33.3	0	0	0	0	0	0	
		3	0	0	0	0	0	0	0	0	0	0	
Total	69	0–1	35	50.7	20	28.9	0	0	0	0	1	2.9	
		2–3	34	49.3	23	33.3	3	8.8	0	0	4	11.8	

¹stages of pathological lesions (PL): 0 (tissues with no lesions), 1 (lymphadenopathy or non-specific lesion in the tissue of parenchymatous organs), 2 (caseation), and 3 (calcification)

²histological examination after the hematoxylin-eosin staining: ± (tissues with lesions like pyogranulomas, lymphadenitis, hyperplasia and abscess), + (granulomatous inflammation)

³IS6110 insertion sequence specific for all members of *Mycobacterium tuberculosis* complex detected by high sensitive PCR described previously (Cave et al., 1991; Bartos et al., 2006)

⁴lymph nodes

⁵two samples of lung tissue

⁶two samples of lung tissue and two samples of liver

Part 3. Abattoir meat inspection

Pathomorphological examination

The meat inspection surveillance carried out during July and August 2005 detected 3.5% of 984 inspected cattle with tuberculous lesions (PL stages 2 and 3). Tuberculous lesions were observed in 25 (52.1 %) of 48 lung ln and in 5 (33.3%) of 15 mesenteric ln (PL stages 2 and 3) and in 4 (66.7%) of 6 the lung and liver organs [PL stage 2 (Table 3)]. Among these, there were 3 lung ln and 2 mesenteric lns from five of nine animals reacting for tuberculin skin test.

Histopathological examination

HE staining. From the 69 histopathologically examined tissue sections, 20 (28.9%) acute lymphadenitis and reactive hyperplasia and 23 (33.3%) pyogranulomas were observed in lung and mesenteric ln, including lung and liver tissues. Abscess and lipomatosis were each found in 1 (1.4%) mesenteric ln. Chronic focal purulent inflammatory reactions due to parasites were evident in 3 (4.3%) tissues. The typical tuberculous lesions, granulomas with calcification, and multinucleated giant cells of Langhans type were detected in 2 (4.2%) and 1 (6.7%) lung and mesenteric ln, respectively. Between tuberculous lesions of lung and mesenteric ln statistically significant ($P < 0.01$) differences were observed (Table 3).

ZN staining. Microscopically no mycobacteria have been detected in all examined tissue samples stained by ZN staining technique (Table 3).

Examination by IS6110 PCR

IS6110 PCR revealed a positive result in 3 (6.3%) and 2 (13.4%) lung and mesenteric ln respectively, among which four tissue samples were with and one with out tuberculous lesions (Table 3).

DISCUSSION

The Addis Ababa abattoir is the largest abattoir found in the country and provides daily beef requirements to the city. Most often in the abattoir, cattle, sheep and goats, coming from different directions of the country, are slaughtered. In the retrospective analysis, tuberculous lesions were detected in 707 (0.028%) examined animals and cattle

were predominantly found with tuberculous lesions than other slaughtered animals (Table 1).

Although the detection rate of tuberculous lesions have showed an increasing trend in recent years (especially from 2003–2005), the rate of positive incidences has been found to be low, considering the quantity of slaughtered animals (Table 1). This result strengthen our suggestion that, in most cases, *post mortem* surveillances, for detection of bovine tuberculous lesions in particular depend on the work load, time and the diligence of the inspector conducting the examination (Corner et al., 1990). Despite this, detection of tuberculous lesions in abattoirs can be affected due to early infection or like in our study parasites, non-specific reactions and due to infection other than *M. bovis* (Corner, 1994) and other irregularities of abattoir meat inspections (Edwards et al., 1997). However, the increasing trend of tuberculous lesions, particularly in the years (2003–2005) confirms that bovine tuberculosis is an existing problem in Ethiopia which needs to be solved.

The tuberculin skin test analysis conducted for two years showed that, herd prevalence is significantly higher (48.2%) than prevalence in individual cattle (18.7%; Table 2). This may be related to the number of animals that attribute to the transmission of the infection, as the rate of transmission of bovine tuberculosis is affected by herd size (O'Reilly and Daborn, 1995). Data concerning age and sex of skin tested animals were not available and thus logistic regression analysis could not be done. The difference in prevalence rates among farms is most likely affected by the production and/or management system, in which the animals kept (Barwineck and Taylor, 1996).

The intensity and distribution of the infection can also affected by breed types. A different susceptibility have been observed even within the local breeds (Mbororo zebu and the Arab zebu cattle in Chad (Diguimbaye-Djaibe et al., 2006b), and in Cameroon (Njanpop-Lafourcade et al., 2001). In a very recent study on the impact of cattle husbandry on the pathology of bovine tuberculosis, Ameni et al. (2006) demonstrated that Holstein cattle kept in doors have produced significantly higher IFN- γ levels in response to avian and bovine PPD than Holstein kept in pasture. Hence, the tuberculin skin test analysis confirmed that, intensive systems favoring close contact between animals facilitate the spread of tuberculosis and other respiratory diseases in comparison to exten-

sive systems, where frequent animal movement is practiced. The discrepancy between the results observed in cattle at slaughterhouses (which most of them were local breeds; Table 3) and the tuberculin skin test analysis in Addis Ababa dairy farms (exotic breeds; Table 2) strengthens our suggestion that, exotic dairy cattle are more susceptible than local zebu breeds and even cross breeds (Kiros, 1998; Regassa, 2005).

The abattoir meat inspection surveillance revealed tuberculous lesions in 3.5% of the total number of inspected animals (PL stages 2 and 3). This was higher than previous finding by various authors where lower prevalence rates have been reported (Nsengawa and Otaru, 1987; Radostits et al., 2000; Asseged et al., 2004). On the contrary, our finding was lower than a study report of Ameni and Wudie (2003), where the prevalence rate was 5.2%. In our study (Table 3) the tuberculous lesions were detected predominantly in the lung and associated ln, which was consistent with the previous findings (Corner et al., 1990; Whipple et al., 1996; Asseged et al., 2004). The current study has showed a higher prevalence rate than the previous data from 1996 to 2005 (Table 1), is related to the presence of difference in number of examined animals in large and the presence of few culled cattle which were reactors for tuberculin skin test.

A significant difference ($P < 0.01$) between the respiratory and mesenteric ln with lesions may be associated with the route of infection. However, observation of tuberculous lesions in 5 (33.3%) mesenteric ln may also imply that, the occurrence of intestinal tuberculosis might have been more frequent than assumed. Although gross lesions were observed in the lungs, typical TB lesions were not detected histopathologically and *MTC* members were not detected by PCR (Table 3). This finding is in contrast to previous result (Whipple et al., 1996), where *M. bovis* was isolated from lungs with out gross lesions.

In most cases, tuberculous lesions in beef cattle in particular may not always be observed, therefore the presence of tuberculous lesions or absence of visible lesions may not often lead to *M. bovis* detection, as isolation of *M. bovis* has been confirmed from non visible lesions (de Kantor et al., 1987; Corner, 1994; Whipple et al., 1996). The presence of caseous and/or calcified lesions and even lesions resembling tuberculous lesions may not always found to be of mycobacterial origin. In contrast, they can be caused by any other intracellular organ-

isms or parasites that could mislead a veterinarian to consider the non tuberculous cattle as being tuberculous. However, during examination of mycobacterial infections, care should be given because of the tuberculous lesions caused by species of mycobacteria not belonging to *MTC*; i.e. *M. fortuitum* and *M. farcinogenes* have been also isolated from cattle and human in Chad (Diguimbaye-Djaiibe et al., 2006a,b). Despite the irregularities and some casual problems we faced during inspection in abattoirs; these findings strengthens our suggestion that abattoir meat inspection is the utmost obligatory and fundamental step in detection of tuberculous cattle and/or in diagnosing of bovine tuberculosis in Ethiopia, where other diagnostic options are limited.

In our histopathological examination, a reactive hyperplasia of follicles (28.9%), acute lymphadenitis and non-specific reactions with pyogranulomas (33.3%) were evident in most sections of ln samples. In some tissue samples parasitic cysts have also been observed. The presence of reactive hyperplasia of lymphatic follicles represents generally the response of the immune system to any antigenic stimulation. The pyogranulomas were composed of central necrosis with the presence of degenerated and/or decayed neutrophilic and eosinophilic granulocytes.

Granulomatous lesions typical to bovine tuberculosis, manifesting granulomas with central necrosis surrounded by multinucleated cells of Langhans cells, were observed only in 3 (4.3%) of the 69 tissue samples in the ln which have had gross lesions. Consistent to this finding, Whipple et al. (1996) described that histologically, manifestation of typical granulomatous lesions in tissues with gross lesions was evident. These granulomas were presented by a central caseation and focal calcifications (Rhyhan and Saari, 1995). Around the necrotic mass a margin formed by large macrophage cells with light cytoplasm was also evident.

Mycobacteria were neither detected by ZN nor HE staining methods in all tissues with lesions. This finding was consistent with a previous report (Gracey, 1986). This result justifies that viable mycobacteria may not be present in calcified lesions or the lesions were caused by infection with other intracellular causal agents and/or parasites. *M. bovis* are often low in bovine specimens and they can be visualized by ZN only if a limited quantity (at least 5×10^4 mycobacteria/ml) of materials is present (Quinn et al., 1994). Despite this, our

result of ZN staining may also be affected by the sample taking technique during smear preparation as mycobacteria are not evenly distributed in the tissue sample. Berk et al. (1996) described that out of 18 PCR positive samples fixed with formaldehyde solution, ZN staining was positive only in three samples, which agrees with our result (0% of 5 PCR positives) and a positive acid fast bacilli staining in a formalin fixed tissue may be most likely low. However, lesions determined to be tuberculous upon examination of HE-stained section with no AFB may be regarded as suggestive of tuberculosis (Thoen et al., 1995).

The IS6110 PCR examination of tissues detected the presence of IS6110 specific for *MTC* in 5 of the 69 (7.2%) cases (Table 3). This finding is consistent with the previous study (Wards et al., 1995), in which 9.2% of 52 tissues samples recovered from *post mortem* examination were positive by PCR. We failed to detect the presence of IS6110 insertion element specific for *MTC* members from tissues with classical tuberculous lesions. The failure of detecting mycobacteria or false-negative results in these samples may be related to factors such as: the low number of mycobacteria present in the sample in particular *M. bovis* infections frequently have low numbers of bacteria and are not evenly distributed through out the body or even within a single ln (Thomson, 2006); due to the presence of PCR inhibitors that limit the efficiency of PCR detection, inefficient extraction of mycobacterial DNA and cross-contamination. In this case the more potential reason may be due to prolonged formalin fixation which progressively increases DNA degradation that eventually leads to false-negative results (Srinivasan et al., 2002). However, in this study the PCR test was efficient in detecting the presence *MTC* members that enables us to make a definitive diagnosis.

CONCLUSIONS

In Ethiopia, the endemic nature of infection due to *M. bovis* has long been reported. Moreover, recent studies showed that *M. bovis* was found to be the cause of cervical lymphadenitis in humans is indicating the significance of bovine tuberculosis in between human and cattle. In addition, the existing Ethiopian eating culture (eating of raw meat and drinking of raw milk), the very common close contact of animals with humans (most common in

rural areas) and the prevailing low standard of hygienic status in the production farms are potential risk factors that favors the spreading of infection. Hence, an immediate and appropriate control program against bovine tuberculosis infection should be designed. Equally it is highly important to integrate research and disease surveillance programs in-between the medical and veterinary institutions, as it helps and simplifies the designing of feasible control program against bovine tuberculosis infection and to minimize zoonotic threat of tuberculosis.

Acknowledgment

We thank Dr. Dagninet Yimenu (MoA, Animal Health Department, Ethiopia) for the continuous transportation support in due course of the study and Dr. Adgollgn Mitiku (Debre-Zeit ELFORA export abattoir, Ethiopia) for providing available data. All meat inspection staffs at Addis Ababa abattoir, for their kind collaboration during the study period, Ing. Ludmila Faldikova for her English translation (part of statistical analysis), Mgr. Maria Vass (Swinburne University of Technology, Victoria, Australia) and Catherine Murdoch (Aberdeen University, Scotland, UK) for critical grammatical correction of the manuscript, RNDr. Milan Bartos, PhD. for critical comments to IS6110 PCR, and Mrs. Dana Svobodova from VRI Brno (Czech Republic) for the laboratory assistance in this work are highly acknowledged.

REFERENCES

- Abdo J. (1993): The epidemiology and control of bovine tuberculosis in Ethiopia. [MSc. Dissertation.] Center for Tropical Veterinary Medicine, University of Edinburgh, UK.
- Ameni G. (1996): Bovine tuberculosis: evaluation of diagnostic tests, prevalence and zoonotic importance. [DVM Thesis.] Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia.
- Ameni G., Wudie A. (2003): Preliminary study on bovine tuberculosis in Nazareth municipality abattoir of central Ethiopia. Bulletin of Animal Health and Production in Africa, 51, 125–132.
- Ameni G., Aseffa A., Engers H., Young D., Hewinson G., Vordermeier M. (2006): Cattle Husbandry in Ethiopia is a predominant factor for affecting the pathology of

- bovine tuberculosis and gamma interferon responses to mycobacterial antigens. *Clinical and Vaccine Immunology*, 13, 1030–1036.
- Ashford D.A., Whitney E., Raghunathan P., Cosivi O. (2001): Epidemiology of selected mycobacteria that infect humans and other animals. *Scientific and Technical Review, Office of International Epizootics*, 20, 325–337.
- Asseged B., Woldesenbet Z., Yimer E., Lemma E. (2004): Evaluation of Abattoir Inspection for the diagnosis of *Mycobacterium bovis* infection in cattle at Addis Ababa Abattoir. *Tropical Animal Health and Production*, 36, 537–546.
- Ayele W.Y., Neill S.D., Zinsstag J., Pavlik I. (2004): Bovine tuberculosis: an old disease but a new threat to Africa. *International Journal of Tubercle and Lung Diseases*, 8, 924–937.
- Bartos M., Pavlikova H., Dvorska L., Horvath R., Dendis M., Flodr P., Kolar Z., Weston R.T., Pac L., Matlova L., Pavlik I. (2006): Risk assessment of mycobacterial infections (human tuberculosis and avian mycobacteriosis) during anatomical dissection of a cadavers. *Veterinarni Medicina*, 51, 320–332. <http://www.vri.cz/docs/vetmed/51-5-311.pdf>
- Barwineck F., Taylor N.M. (1996): Bovine tuberculosis assessment of the socio-economic importance of tuberculosis in Turkey and possible strategies for control or eradication. *Ankara. Eschborn Deutsche Gesellschaft fur Technische Zusammenarbeit*, 3–45.
- Berk R.H., Yazici M., Atabey N., Ozdamar O.S., Pabucoglu U., Alici E. (1996): Detection of *M. tuberculosis* in formaldehyde solution-fixed, paraffin-embedded tissue by polymerase chain reaction in Pott's disease. *SPINE*, 21, 1991–1995.
- Boddinghaus B., Rogall T., Blocker H., Botteger E.C. (1990): Detection of mycobacteria by amplification of rRNA. *Journal of Clinical Microbiology*, 28, 1751–1759.
- Cave M.D., Eisenach K.D., McDermott P.F., Bates J.H., Crawford J.T. (1991): IS6110 Conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. *Molecular and Cellular Probes*, 5, 73–80.
- Corner L.A. (1994): *Post mortem* diagnosis of *M. bovis* infection in cattle. *Veterinary Microbiology*, 40, 53–63.
- Corner L.A., Melville L., McCubbin K., Small K.J., McCormick B.S., Wood P.R., Rothel J.S. (1990): Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. *Australian Veterinary Journal*, 67, 389–392.
- Cosivi O., Grange J.M., Daborn C.J., Raviglione M.C., Fujikura T., Cousins D., Robinson R.A., Huchzermeyer H.F.A.K., de Kantor I., Meslin F.X. (1998): Tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging Infectious Diseases*, 4, 59–70.
- Cousins D.V., Wilton S.D., Francis B.R. (1991): Use of DNA amplification for the rapid identification of *Mycobacterium bovis*. *Veterinary Microbiology*, 27, 187–195.
- Daborn C.J., Grange J.M., Kazwala R.R. (1996): The bovine tuberculosis cycle: an African perspective. *Journal of Applied Bacteriology*, 81, Suppl., S27–S32.
- de Kantor I.N., Nader A., Bernardelli A., Giron D.O., Man E. (1987): Tuberculosis infection in cattle not detected in slaughterhouse inspection. *Journal of Veterinary Medicine*, 34, 202–205.
- Diguimbaye-Djaibe C., Hilty M., Ngandolo R., Mahamat H.H., Pfyffer G.E., Baggi F., Hewinson G., Tanner M., Zinsstag J., Schelling E. (2006a): *Mycobacterium bovis* isolates from tuberculous lesions in Chadian Zebu Carcasses. *Emerging Infectious Diseases*, 12, 769–771.
- Diguimbaye-Djaibe C., Vincent V., Schelling E., Hilty M., Ngandolo R., Mahamat H.H., Pfyffer G.E., Baggi F., Tanner M., Zinsstag J. (2006b): Species identification of non-tuberculous mycobacteria from humans and cattle of Chad. *Schweizerisches Archiv fur Tierheilkunde*, 148, 251–256.
- Edwards D.S., Johnston A.M., Mead G.C. (1997): Meat inspection: an over view of present and future trends. *The Veterinary Journal*, 154, 135–147.
- Enarson D.A. (2006): Introduction. In: Thoen C.O., Steele F.H., Gilsdorf M.J. (eds.): *Mycobacterium bovis* Infection in Animals and Humans. 2nd ed. Iowa State University Press, Ames, Iowa, USA. 1–5.
- Erler W., Martin G., Sachse K., Naumann L., Kahlau D., Beer J., Bartos M., Nagy G., Cvetnic Z., Zolnir-Dovc M., Pavlik I. (2004): Molecular fingerprinting of *Mycobacterium bovis* subsp. *caprae* isolates from Central Europe. *Journal of Clinical Microbiology*, 42, 2234–2238.
- Flesja K.I., Fodstad F., Ulvesaeter H.O. (1978): Tuberculous lesions in lymph nodes of swine (in Norwegian). *Nordisk Veterinary Medicine*, 30, 71–73.
- Gracey J.F. (1986): *Meat Hygiene*. 8th ed. Bailliere Tindall, London. 350–360.
- Hailemariam S. (1975): A brief analysis of activities of Meat Inspection and Quarantine Division, Department of Veterinary Service, Ministry of Agriculture, Addis Ababa, Ethiopia.
- Kazwala R.R., Daborn C.J., Sharp J.M., Kambarage D.M., Jiwa S.F.H., Mbembati N.A. (2001): Isolation of *M. bovis* from human cases of cervical lymphadenitis in Tanzania: a cause of concern? *International Journal of Tubercle and Lung Diseases*, 5, 87–91.

- Kent P.T., Kubica G.P. (1985): Public Health Mycobacteriology: a guide for the level III laboratory. US Department of Health and Human Services, Public Health Service, Centers for Disease Control Atlanta, Georgia, USA. 207 pp.
- Kidane D., Olobo J.O., Habte A., Negesse Y., Aseffa A., Abate G., Yassin M.A., Bereda K., Harboe M. (2002): Identification of the causative organism of tuberculosis lymphadenitis in Ethiopia by PCR. *Journal of Clinical Microbiology*, 40, 4230–4234.
- Kiros T. (1998): Epidemiology and importance of bovine tuberculosis in selected sites of Eastern Shewa, Ethiopia. [MSc. Thesis.] Faculty of Veterinary Medicine, Addis Ababa University and Freie University of Berlin, 65 pp.
- Lesslie I.W., Brin K.J. (1970): *Mycobacterium avium* infections in cattle and pigs in Great Britain. *Tubercle*, 51, 446–451.
- Matouskova O., Chalupa J., Cigler M., Hruska K. (1992): STAT Plus – Manual (in Czech). 1st ed. Veterinary Research Institute, Brno. 168 pp.
- Neill S.D., Pollock J.M., Bryson D.B., Hanna J. (1994): Pathogenesis of *Mycobacterium bovis* infection in cattle. *Veterinary Microbiology*, 40, 41–52.
- Njanpop-Lafourcade B.M., Inwald J., Ostyn A., Durand B., Hughes S., Thorel M.F., Hewinson G., Haddad N. (2001): Molecular typing of *Mycobacterium bovis* isolates from Cameroon. *Journal of Clinical Microbiology*, 39, 222–227.
- Nsengawa G.R., Oturu M.M. (1987): Prevalence of TB in the dairy farms in southern Tanzania. *Bulletin of Animal Health and Production in Africa*, 35, 251–252.
- O.I.E. (2000): Diagnosis of bovine tuberculosis. In: Manual of Standards for Diagnostic Tests and Vaccines, 267–271.
- Omer E.E., Sanosi S.M., Greafier C.J. (1995): Tuberculin sensitivity in Sudanese population in contact with cattle. *Bulletin of Animal Production in Africa*, 28, 91–96.
- O'Reilly L.M., Daborn C.J. (1995): The epidemiology of *Mycobacterium bovis* infection in animals and man. *Tubercle and Lung Diseases*, 76, 1–46.
- Pavlik I. (2006): The experience of new European Union Member States concerning the control of bovine tuberculosis. *Veterinary Microbiology*, 112, 221–230.
- Pavlik I., Trcka I., Parmova I., Svobodova J., Melicharek I., Nagy G., Cvetnic Z., Ocepek M., Pate M., Lipiec M. (2005): Detection of bovine and human tuberculosis in cattle and other animals in six Central European countries during the years 2000–2004. *Veterinarni Medicina*, 50, 291–299. <http://www.vri.cz/docs/vet-med/50-7-291.pdf>
- Pavlik I., Ayele W.Y., Parmova I., Melicharek I., Hanzlikova M., Körmendy B., Nagy G., Cvetnic Z., Katalinic Jankovic V., Ocepek M., Zolnir-Dovc M., Lipiec M. (2003): *Mycobacterium tuberculosis* in animal and human populations in six Central European countries during 1990–1999. *Veterinarni Medicina*, 48, 83–89. <http://www.vri.cz/docs/vetmed/48-4-83.pdf>
- Pavlik I., Bures F., Janovsky P., Pecinka P., Bartos M., Dvorska L., Matlova L., Kremer K., Van Soolingen D. (2002): The last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995 was caused by *Mycobacterium bovis* subspecies *caprae*. *Veterinarni Medicina*, 47, 251–263. <http://www.vri.cz/docs/vet-med/47-9-251.pdf>
- Pollock J.M., Neill S.D. (2002): *Mycobacterium bovis* infection and tuberculosis in cattle. *Veterinary Journal*, 163, 115–127.
- Popluhar L., Haladej S., Havelka B., Zubaj J. (1974): Occurrence of tuberculosis in husbandry animals in Slovakia in 1972 (in Slovak). *Veterinarstvi*, 24, 33–34.
- Prodinger W.M., Eigentler A., Allerberger F., Schonbauer M., Glawischnig W. (2002): Infection of red deer, cattle, and humans with *Mycobacterium bovis* subsp. *caprae* in western Austria. *Journal of Clinical Microbiology*, 40, 2270–2272.
- Quinn P.J., Carter M.E., Markey B., Carter G.P. (1994): *Mycobacterium* species: In: Clinical Veterinary Microbiology, Wolfe Publishing, London. 156–170.
- Radostits O.M., Gray G.C., Blood D.C., Hincheliff K.W. (2000): A text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. In: Veterinary Medicine. 9th ed. Harcourt Publisher Ltd., London. 909–918.
- Regassa A. (2005): Study on *Mycobacterium bovis* in animals and human in and around Fiche, North Shewa Zone, Ethiopia. [MSc. Thesis.] Faculty of Veterinary Medicine, Addis Ababa University, Debre-Zeit, Ethiopia, 58 pp.
- Rhyan J.C., Saari D.A. (1995): A comparative study of the histopathologic features of bovine tuberculosis in cattle, fallow deer (*Dama dama*), Sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*) and elk. *Veterinary Pathology*, 32, 215–220.
- Schliesser T. (1976): Vorkommen und Bedeutung von Mykobakterien bei Tieren. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, Series A Medical Microbiology Infectious Diseases, Virology, Parasitology*, 235, 184–194.
- Srinivasan M., Sedmak D., Jewell S. (2002): Effect of fixatives and tissue processing on the content and integrity of nucleic acid. *American Journal of Pathology*, 161, 1961–1971.
- Thoen C.O., Karlson A.G., Himes E.M. (1981): Mycobacterial infections in animals. *Review Infectious Diseases*, 3, 960–972.

- Tohen C.O., Huchzermeyer H., Himes E.M. (1995): Laboratory diagnosis of bovine tuberculosis. In: Tohen C.O., Steele J.H. (eds.): *M. bovis* Infection in Animals and Humans. Iowa State University Press, Ames, Iowa, USA. 63–72.
- Thomson B. (2006): Polymerase Chain Reaction detection of *Mycobacteria tuberculosis* complex in formalin fixed tissues. In: Tohen C.O., Steele, F.H., Gilsdorf M.J. (eds.): *Mycobacterium bovis* Infection in Animals and Humans. 2nd ed. Iowa State University Press, Ames, Iowa, USA. 63–67.
- Thrusfield M. (1995): Diagnostic testing. In: Veterinary Epidemiology. 2nd ed. Black Well Science Ltd., Cambridge, USA. 225 pp.
- Wards B.J., Collins D.M., de Lisle G.W. (1995): Detection of *Mycobacterium bovis* in tissues by polymerase chain reaction. *Journal of Veterinary Microbiology*, 43, 227–240.
- Whipple D.L., Bolin C.A., Miller J.M. (1996): Distribution of lesions in cattle infected with *M. bovis*. *Journal of Veterinary Diagnostic and Investigation*, 8, 351–354.
- Zinsstag J., Kazwala R.R., Cadmus I., Ayanwale L. (2006): *Mycobacterium bovis* in Africa. In: Tohen C.O., Steele, F.H., Gilsdorf M.J. (eds.): *Mycobacterium bovis* Infection in Animals and Humans. 2nd ed. Iowa State University Press, Ames, Iowa, USA. 199–210.
- Zumla A., Squire S.B., Chintu C., Grange J.M. (1999): The tuberculosis pandemic: implications for health in the Tropics. *Trans Royal Society for Tropical Medicine and Hygiene*, 93, 113–117.

Received: 2006–06–27

Accepted after corrections: 2006–10–18

Corresponding Author:

Prof. MVDr. Ivo Pavlik, CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 601, fax +420 541 211 229, e-mail: pavlik@vri.cz
