International Food Research Journal 21(2): 577-582 (2014)
Journal homepage: http://www.ifrj.upm.edu.my

Bovine tuberculosis survey based on meat inspection and microscopic examination in central city abattoir in Ismailia, Egypt and its hazards to the abattoir workers

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Article history

Received: 1 September 2013
Received in revised form: 14 October 2013
Accepted: 15 October 2013

Keywords

Bovine tuberculosis
Abattoir
Buffalo
Zoonoses

Abstract

This article aimed to investigate bovine tuberculosis in cattle and buffalo slaughtered in Ismailia city abattoir, Egypt and examining abattoir workers for tuberculosis infection. Carcasses were examined for tuberculosis-like lesions for one year starting from March 21st 2009. Tuberculosis-like lesions were examined by Ziehl-Neelsen staining and histopathological techniques. Results revealed that of 13,866 slaughtered bovine, tuberculosis-like lesions were detected in 86 (0.6%) of carcasses including 2 (2.3%) generalized and 84 (97.7%) localized lesions. Microscopic examination for Acid-Fast Bacilli (AFB) and histopathological examination showed that 38 (0.3%) carcasses and 50 (43.9%) of tuberculosis-like lesions were confirmed to be tuberculosis. Lesions in the lung and its associated lymph nodes were the most predominant (64.8%). The tuberculosis-like lesions were grossly detected to be higher among cattle (0.7%) than buffalo (0.5%). There were significant differences between male and female in the detection rate of tuberculosis-like lesions ($P < 0.0007$), however, seasonal effects were not significant. Tuberculin Intradermal testing of the abattoir workers revealed 12.5% of them were reactors whereas all were negative to AFB by microscopic examination of their sputum smears stained by Ziehl-Neelsen. In conclusion, the detection of bovine tuberculosis among the slaughtered bovine indicated its spread among the household animal husbandry in Ismailia, Egypt that should be covered by national control programs to prevent zoonotic potentials of bovine tuberculosis.

Introduction

Bovine tuberculosis (BTB) is a chronic infectious disease caused by *Mycobacterium bovis*. This disease is widely distributed throughout the world and mainly affects animals with occasional human involvement (O'Reilly and Daborn, 1995). Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection in cattle, but infection by ingestion of contaminated material may also occur (Cousins, 2001). However, *M. bovis* infection in humans can occur through the consumption of contaminated raw or undercooked dairy and/or meat products; meanwhile occupational infection may occur due to exposure through airborne infection among farmers, veterinarians, and slaughterhouse workers (Cousins and Dawson, 1999; Biet *et al.*, 2005; Michel *et al.*, 2010). The proportion of tuberculosis induced by *M. bovis* among humans is relatively low compared to *M. tuberculosis*. However, *M. bovis* tuberculosis has become increasingly prevalent among human populations subjected to poverty, malnutrition, human immunodeficiency virus, and inadequate health care (Michel *et al.*, 2010). The global prevalence of human tuberculosis caused by *M. bovis* has been estimated to be 3.1% of all tuberculosis cases, accounting for 2.1% and 9.4% of pulmonary and extra pulmonary tuberculosis cases respectively (Cosivi *et al.*, 1998).

In Africa, the BTB is widespread and is affecting the animal industries and human health, posing serious public health threats (Cosivi *et al.*, 1998; Ayele *et al.*, 2004; Thoen *et al.*, 2009). Africa is assumed to bear the highest consequences of zoonotic TB worldwide because of the frequent and concurrent presence of multiple risk factors (Müller *et al.*, 2013). In Egypt, the trend in the annual risk of *M. bovis* infection of human has decreased in the last decades (El-Ibiary *et al.*, 1999). A prevalence of 12.2% of the total number of human tuberculosis cases in 1953, falling to 10 % in 1969, and 5.4% in 1980 has been reported (Cosivi *et al.*, 1998). *M. bovis* has been identified in nine of 20 randomly-selected mycobacteria samples isolated from patients with abdominal tuberculosis (Nafeh *et al.*, 1992). In addition, *M. bovis* has been identified in the cerebrospinal fluid of a pateint suffering from tuberculous meningitis (Cooksey *et al.*, 2002). In animals, BTB has been reported in cattle and buffalo in Egypt since 1920, when the overall rate of infection in the cattle and buffalo population was estimated at 2-9% by tuberculin testing. This prevalence dropped

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to 2.6% in 1985 as a result of establishment of national program which started in 1981 (Cosivi *et al*., 1998). The prevalence of BTB of cattle from different farms was 1.9% by tuberculin intradermal test (Mosaad *et al*., 2012). Moreover, zoonotic infections of BTB among dairy farm workers has been reported (Hassanian *et al*., 2009).

Identification of *M. bovis* by culture and biochemical methods is important for definitive diagnosis (Corner, 1994). However, because of the technical problems and cost, they have not come into widespread use in veterinary diagnostic laboratories (Araujo *et al*., 2005). Most of the abattoirs in Egypt do not have diagnostic facilities for rapid confirmation of grossly detected BTB lesions. In addition, regular testing of millions of individual cattle for surveillance of BTB by the tuberculin skin test could not include all animals, especially at the household sector. Therefore, abattoir inspection remains an affordable technique for monitoring the prevalence of BTB in bovine in Egypt. The objectives of this study were to determine the prevalence of BTB among the bovine slaughtered in Ismailia city abattoir, Ismailia, Egypt, and to assess the zoonotic implications on the abattoir workers.

Materials and Methods

Study area

This study was done at the central city abattoir located in Abo-Atwa district of Ismailia, Egypt. It is one of 10 abattoirs in Ismailia government; however, its capacity is 65% of total slaughtered cattle and buffalo. Ismailia city is located along the Suez Canal, Egypt, 140 km at north-east of Cairo.

Animals under study

According to the general authorities of veterinary medicine, Ismailia branch, the total number of cattle and buffalo in Ismailia governorate in 2010 was estimated to be 54,920 and 29,050 respectively. A total of 16,244 bovine animals, including 10,055 cattle, 3,811 buffalo and 2,378 male buffalo calves (slaughtered for veal meat) were slaughtered and inspected for one year from March 21st, 2009 (the beginning of spring season). Of 13,866 adult bovines, 13,049 (94.1%) were fattening bulls aged from 2-4 years, divided into 9,880 cattle bulls and 3169 buffalo bulls. A total of 817 (5.9%) were cows, including, 175 cattle cows and 642 buffalo cows. All the cows were aged over five years, and all animals were owned in household production.

Inspection of bovine carcasses for detection of Tuberculosis-like lesions

The carcasses of bovine animals slaughtered were routinely examined according to procedure of Egyptian guidelines for inspection of cattle (EOS 517 1986). Samples of caseated, suppurative granulomatous lymph nodes and granuloma from parenchymatous organs were collected in polyethylene bags and quickly delivered to the laboratory in ice. Meat inspection was carried out at the Ismailia abattoir by well-trained veterinarians under close supervision of local authority.

Microscopic pathological lesion

A part of each lesion from meat and organs was fixed in 10% formalin and the sections were prepared by the paraffin embedding technique according to Banchroft *et al.* (1996). Paraffin sections were cut at five microns in thickness and stained with hematoxylin and eosin stain followed by microscopic examination.

Staining of tissue with Ziehl-Neelsen stain

Collected specimens from each macroscopic tuberculosis-like lesion were examined for the presence of AFB using Ziehl-Neelsen according to Wentworth (1987). Direct smear films were prepared from tissue exudates. Histopathological control positive slides showed multiple granulomas and Ziehl-Neelsen stained smears showed acid fast bacilli of lymph node from cattle confirmed to be *M. bovis* by bacteriological culturing and real-time PCR assay.

Tuberculin testing and direct AFB staining in sputum of the abattoir workers

The abattoir workers were admitted to Ismailia Chest Hospital as outpatients to check for indications of tuberculosis. 0.1 ml of 5U-strength purified protein derivative (VACSERA, Cairo, Egypt), a cell-free purified protein fraction obtained from a human strain of *M. tuberculosis*, was intradermally injected according to the Mantoux technique on the dorsal surface of the left forearm. The reaction was read between 48 and 72 h after administration. It was considered positive if the injection was followed by induration of 10 mm or more in diameter (Sinder, 1982).

Sputum smears were collected from the abattoir workers by deep coughing on getting up in the morning and the smears were stained by Ziehl-Neelsen stain as mentioned above. Questionnaire was directed to the abattoir workers and veterinarian for history of chronic coughing and skin lesions suspected to tuberculosis.

Data analysis

The significance of gender and seasons on the prevalence of bovine with tuberculosis-like lesions among cattle and buffalo species was determined using a Chi-square contingency with Fisher's exact test (two tailed). Statistics were computed using GraphPad Prism (Version 5) software. P value of <0.005 was considered statistically significant.

Results

Total prevalence of tuberculosis-like lesions and effects gender and seasons

As tabulated in Table 1, of 13,866 cattle and buffalo carcasses inspected during the survey in Ismailia abattoir, 86 (0.6%) had tuberculosis-like lesions; 2 (2.3%) were generalized TB, and 84 (97.7%) were localized TB restricted to lymph nodes and parenchymatous organs. The two generalized tuberculosis were a cattle cow and a buffalo cow, their ages were above five years. Tuberculosis-like lesions were detected in 0.7% of cattle which was higher than in buffalo (0.5%). Based on gender, results revealed that cows showed highly significant ($P < 0.007$) prevalence (2.6%) compared to bulls (0.5%) among cattle and buffalo. Moreover, there was no significant effect of seasons on the prevalence of BTB detection among cattle and buffalo.

Frequencies and localization of tuberculosis-like lesions examined by Ziehl-Neelsen staining and histopathological techniques

As shown in table 2, out of 13,866 carcasses inspected in this study, 38 carcasses (0.3%) were confirmed to have tuberculosis by microscopic examination for Acid Fast Bacilli (AFB) and histopathological examination. Tuberculosis was generalized in two carcasses (5.3%) and localized in 36 (94.7%) carcasses.

The microscopically positive lesions for AFB were positive in 50 (43.9%) of 114 grossly detected tuberculosis-like lesions. It was distributed by a prevalence of 58.3%, 37.3%, 40%, and 64.7% in head, lung, liver/intestine and udder respectively. The frequency percentages of localized tuberculosis in the head, lung, liver/intestine and udder to the total number of lesions were (10.5%), (64.8%), (8.8%) and (14.9%) respectively.

Table 1. Prevalence of tuberculosis-like lesions among inspected cattle and buffalo and effects of gender and seasons

Variables	Cattle		Buffalo		Total		Significance Chi square
	No.	+ve (%)	No.	+ve (%)	No.	+ve (%)	
Total prevalence	10055	66 (0.7%)	3811	20 (0.5%)	13866	86 (0.6)	
Gender							
Bulls	13049	65 (0.5%)	9880	56 (0.6%)	3169	9 (0.3%)	$P < 0.007$
Cows*	817	21 (2.6%)	175	10 (5.7%)	642	11 (1.7%)	
Season							
Autumn	3574	25 (0.7%)	2539	19 (0.7%)	1035	6 (0.6%)	
Winter	2494	15 (0.6%)	1762	12 (0.7%)	732	3 (0.4%)	$P < 1.0$
Spring	2897	25 (0.9%)	2025	19 (0.9%)	872	6 (0.7%)	
Summer	4901	21 (0.4%)	3729	16 (0.4%)	1172	5 (0.4%)	

* The fattening bulls of age 2 years or more were allowed to be slaughtered in the abattoir, however; only elderly cows (above 5 years) and females in emergency were allowed for slaughtering

Table 2. Localization and frequencies of tuberculosis-like lesions examined by direct acid-fast staining and histopathology

Location of lesions	Gross TB -like lesions N=114	AFB/Histopathology
	+ve (%)	+ve/tested (%)
Head	12 (10.5%)	7/12 (58.3%)
Lung	75 (64.8%)	28/75 (37.3%)
Liver/Intestine	10 (8.8%)	4/10 (40%)
Udder	17 (14.9%)	11/17 (64.7%)
Total lesions (N = 114)		50/114 (43.9%)
Total lesioned animals*		38/86 (41.9 %)

*Including two generalized tuberculosis cases

Histopathological examination

Histopathological section in lymph node showed multiple granuloma with caseated center surrounded by a layer of epithelioid macrophages, neutrophils and multinucleated giant cells. Histopathological sections in lung showed focal aggregation of lymphocytes and macrophages with presence of Langhan's giant cell.

Abattoir workers examination for tuberculosis

By Tuberculin Intradermal test, 2 out of 16 (12.5%) of the abattoir workers were reactors, while all were negative for AFB by Ziehl-Neelsen staining of sputum smears. None of the abattoir workers had chronic coughing or skin lesions to be suspected as tuberculous lesions.

Discussion

Adequate palpation of lesions at postmortem with production of gritty sound incisions is the basis of tentative diagnosis of BTB. This form of diagnosis, though unconfirmed, helps to a great extent in reducing the extent of the disease in cattle and its potentials of spreading to humans and other animals (Corner, 1994). In this study, the prevalence of tuberculosis was 0.6% among bovine slaughtered animals at Ismailia abattoir based on grossly detected tuberculosis-like lesions. This finding was much lower than earlier reports based on gross lesions detection on abattoir in different governorates

(Adaway, 1986). In addition, Hassanian *et al.* (2009) detected an incidence of BTB of 58.7% in one dairy farm by intradermal tuberculin testing. Mosaad *et al.* (2012) reported a detection rate of 1.9% in different cattle farms at Nile Delta area. This prevalence was considerably lower than preceding reports in African countries detected by gross examination for which 6% were reported in Sudan (Asil *et al*., 2012) and 19% in Kenya (Gathogo *et al*., 2012). In Ethiopia, varying detection rates based on post-mortem examination in abattoirs have been reported (Shitaye *et al*., 2006; Regassa *et al.*, 2010). Considering the low sensitivity of routine abattoir inspection (Biffa *et al.*, 2010), it is likely that the prevalence in this study is lower than the actual prevalence in this cattle population.

Based on microscopic detection of AFB and histological characteristic of TB, BTB was confirmed in 0.3% of carcasses; nevertheless, it still much higher than the definitive diagnosis by culturing and molecular techniques. The higher prevalence of BTB in cattle compared to buffalo was in consistent with the previous reports (El-Olemy *et al.*, 1985; Cosivi *et al.*, 1998). The percentage of generalized TB detected in this study (2.3%) was much lower compared to numerous abattoir surveys of BTB conducted in different countries, 13.2% (Asil *et al.*, 2012) and 20.5% (Demelash *et al.*, 2009). The two cases of generalized tuberculosis were detected in elderly cows. This result is supported by that previously reported by Regassa *et al.* (2008). Generalized BTB cases were owned by farmers, and they were not formerly tested by tuberculin skin test; consequently, these cases, were also sources of spreading the disease to other animals posing a potential risk of zoonoses. The general authorities of veterinary services in Egypt are following up all bovine farms by regular tuberculin testing and immediate slaughtering of the reactors. However, there are difficulties to cover the household bovines which represent a high proportion of animal production in Egypt. Indeed, programs based on slaughterhouse surveillance are only effective when they use a reliable traceability system for tracing-back to herd of origin that is a difficult measure in owned bovines. In addition, owned cattle had more chances for contacting with human with active tuberculosis that might be a factor for BTB prevalence (Regassa *et al.*, 2010).

In this study, tuberculosis-like lesions were observed predominantly in the lungs and associated lymph nodes (64.8%). This finding was in agreement with the previous reports (Shitaye *et al*., 2006; Reggassa *et al*., 2010; Gathogo *et al*., 2012; Mosaad *et al*., 2012). The distribution and development of lesions depend on the route of transmission (Araujo *et al*., 2005) and location can vary, although most often they are found in thoracic lymph nodes due to infection via the respiratory route (Whipple *et al.*, 1996). Moreover, detection of localized BTB in supra mammary lymph nodes by 14.9% of localized cases were of great public health significance because that poses a potential risk of zoonotic infections of BTB (Hassanian *et al.*, 2009). This finding was much lower than that previously recorded by (58.3%) (Mosaad *et al*., 2012).

In this study, the grossly detected tuberculosis-like lesions were detected in cattle more than buffalo species, which might be related to the predominance of cattle breeding in Egypt than buffalo. A similar finding has been reported by Michel *et al.* (2007). The prevalence of tuberculosis-like lesions was significantly higher in elder cows than bulls which may be contributed to both age and gender factors. This accords with findings by other researchers in Egypt (El-Olemy *et al*., 1985; Mosaad *et al*., 2012) and in other countries (Ameni *et al.*, 2003; Regassa *et al*., 2010). According to the EOS 517 1986, cows are restricted from slaughtering except elderly cows over five years or emergency slaughtering. Regarding seasonal effects, there was no significant effect of seasons on the prevalence of BTB. Generally, the detection rate of meat abnormalities in Egypt could be influenced by the slaughtering rate which elevated during religious feasts and socio-cultural ceremonies. The main risk of human infection with BTB is illegal slaughtering of animals outside the slaughterhouses without inspection by professional well-trained veterinarians.

Human infection by *M. bovis* occurs from ingesting contaminated raw or unpasteurized milk, undercooked meat and inhaling cough spray from infected livestock (Ayele *et al*., 2004; Awah Ndukum *et al.*, 2010; Bifa *et al*., 2011). Meat handlers in developing countries bear high risk to BTB owing to prevailing social and cognition determinants (Hambolu *et al*., 2013). In this study, none of the abattoir workers were positive for microscopic detection of AFB. In a study by Hassanian *et al.* (2009), 40% of farm workers attending a dairy farm with high prevalence of *M. bovis* infections were positive by tuberculin intradermal test and ELISA tests, while their sputum samples were negative for mycobacterial culture. Moreover, El Sabban *et al.* (1992) reported that 5% of 300 mycobacteria cultured from human sputum were *M. bovis* which was attributed to the fact that most of patients were living in Cairo abattoir area, and some were workers in the abattoir. High risk of infection potentials could be caused by of lack of awareness about the mode and risk of BTB infection (Awah Ndukum *et al*., 2010; Ibrahim *et al*., 2012). Therefore, regular health check of the abattoir workers for occupational infections by tuberculosis, and implementation of awareness programs is important.

In conclusion, detection of BTB among the slaughtered animals indicates the presence of BTB in the Egyptian animal husbandry with relevance to human zoonoses. Therefore, proper implementation of meat inspection procedures during slaughtering with public awareness are important to control BTB in Egypt. A large-scale surveillance is needed to estimate the apparent and true prevalence of bovine tuberculosis in Egypt.

Acknowledgments

We would like to thank Dr. Ahmed El-Garhy and Dr Ahmed Farouk, a meat inspector veterinarians at Abo Atwa abattoir, Ismailia Egypt, for their efforts in recording data. We are also very Thankful for Dr Amina A. Dosoki, Dept. of Pathology, Faculty of Veterinary Medicine, Suez Canal University, for her help in the pathological examination of tissue sections.

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