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Zoonotic tuberculosis in occupationally exposed groups in Pakistan

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Background	Bovine tuberculosis (bTB) remains a public health threat in low-income countries. Data on human tuberculosis (TB) due to *Mycobacterium bovis* are poorly documented in developing countries.
Aims	To determine the occurrence of active pulmonary TB due to *M. bovis* in abattoir workers, butchers, livestock farmers and veterinarians and to document their knowledge and practices regarding bTB.
Methods	A cross-sectional study of abattoir workers, butchers, livestock farmers, veterinary doctors and veterinary assistants, which obtained data on socio-demographic conditions, knowledge and practices regarding TB. Sputum samples were collected from respondents with a chronic cough. The chi-squared test was used for statistical analysis.
Results	A total of 141 abattoir workers, 317 butchers, 50 livestock farmers, five veterinary doctors and three veterinary assistants took part. Four out of 16 abattoir workers and 1 out of 50 livestock farmers were positive for *M. bovis* by PCR analysis. Duration of work as an abattoir worker was significantly associated ($P < 0.05$) with prevalence of zoonotic TB. The knowledge of abattoir workers, butchers, livestock farmers and veterinary assistants regarding transmission of bTB from animals to humans and the symptoms of TB in humans was very poor. Most of these workers did not use protective equipment and appropriate safe working techniques and were considered at high risk of acquiring zoonotic TB.
Conclusions	Zoonotic TB is a significant public health issue among professionally exposed groups in Peshawar, Pakistan and suggests a need for further detailed investigations of the disease in this and similar areas.
Key words	*M. bovis*; occupational exposure; public health; zoonotic tuberculosis.

Introduction

Human tuberculosis (TB) remains a major cause of mortality worldwide and a serious public health threat [1]. The World Health Organization (WHO) estimated that in 2014, human TB incidence and mortality was 9.6 million and 1.5 million, respectively [2]. The problem has been aggravated by an increase in the incidence of human immunodeficiency virus (HIV) and by the emergence of multidrug resistance [3]. HIV is considered to have a major role in progression to active TB among those individuals infected both with HIV and TB [4]. *Mycobacterium tuberculosis* is the most frequent causative agent of TB in humans. However, humans can also be infected with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB) [5]. Humans acquire *M. bovis* infection by inhalation or ingestion of the bacilli [6]. Control of bTB infection in cattle and pasteurization of dairy products have minimized transmission in developed countries [7,8]. However, it is still an important zoonotic disease in countries where bTB is endemic and pasteurization of milk is not mandatory [9]. Data regarding human TB due to *M. bovis* (zoonotic TB) are poorly documented in developing countries because the diagnosis of TB most often relies on sputum cytology only. Pakistan, with a population of about 190 million, is

ranked fifth among high-TB-burden countries [10]. The WHO has recommended the collection of data about zoonotic TB in developing countries [11].

In Pakistan, animals are not routinely screened for bTB using tests such as the tuberculin skin test. Several independent studies have shown varying prevalence of bTB in cattle and buffalos, e.g. 6% in Peshawar [12], 8% in Lahore [13], 2.5% on various livestock farms in Punjab [14] and 10% at a livestock experimental station in Punjab [15]. Similarly, an estimated 14% prevalence has been reported in buffalo and 13% in cattle at a slaughterhouse in Peshawar [16]. Most of these studies focused on prevalence in cattle and buffalo and no data are available on zoonotic TB occurrence in livestock workers, veterinarians, abattoir workers and butchers or on the risk factors for zoonotic TB in these high-risk groups. Unfortunately, due to lack of currently operational national bTB control and prevention strategies, the trends and status of bTB in Pakistan are not known [1]. Therefore, this study was conducted with the objective of determining the burden of active pulmonary TB caused by *M. bovis* in abattoir workers, butchers, veterinarians and livestock farmers and to document associated risk factors. We also report on the knowledge and practices of these professionals regarding TB.

Methods

This study was conducted in Peshawar district, the capital of Khyber Pakhtunkhwa province, Pakistan, in January and February 2015. The district has an estimated human population of 3.5 million and is situated in a large valley close to the border with Afghanistan. It has two government-administered abattoirs and a few private abattoirs. Several government and private veterinary hospitals and dispensaries provide treatment services to animals in the area. The study participants consisted of abattoir workers from the two government-administered abattoirs, all the butchers from 100 randomly selected meat retail shops, farmers with chronic cough, veterinary assistants and veterinarians. The abattoir heads met with the abattoir workers to explain the aim and methodology of the study and workers who agreed to participate were included in the study. A convenience sample of 50 livestock farmers from the study area who were coughing up sputum or blood were included in the study. The farmers were identified by conducting door to door visits of farmers' households and inquiring about the presence of a farmer with cough in the household from family members. All veterinarians and veterinary assistants working in the area were contacted via mobile phone and those who had sputum or blood along with cough were included in the study.

Interviews were conducted by one author (I.K.) in the local language (Pushto) and used a pre-designed questionnaire which focused on socio-demographic conditions and on knowledge and practices concerning TB. Separate questionnaires were developed for abattoir workers, butchers, veterinarians/veterinary assistants and farmers.

Sputum samples were processed in the Provincial Tuberculosis Reference Laboratory (Biosafety level III), Peshawar. DNA was isolated from sputum using a Genolyse® DNA kit and the PCR protocol described by Tipu *et al.* [17] for detection of *M. bovis* was followed with some modifications. Data were processed and analysed with SPSS (version 15.0) and Microsoft Excel (2010). Chi-square with 95% confidence intervals was calculated to assess statistical significance. Frequencies and percentages were used to present the findings. The study was approved by the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore.

Results

The study participants consisted of 141 abattoir workers, 317 butchers, 50 farmers, five veterinary assistants and three veterinarians. The response rate of the abattoir workers was ~90% (141/156), and all were male. Of 141 abattoir workers interviewed, 16 had chronic cough and we obtained sputum samples for detection of *M. bovis* (see Table 1). Of these, four were positive for *M. bovis* by PCR. Duration of work as an abattoir worker was significantly associated ($P < 0.05$) with occurrence of zoonotic TB. Those who had worked for >15 years in the abattoir were more affected by zoonotic TB than those who worked for a shorter duration. Age, education and type of work carried out by workers were not significantly associated with zoonotic TB (Table 2). Nineteen abattoir workers (14%) were involved in live animal

Table 1. Distribution of samples and PCR results

Group	Total number in study area	Number interviewed	Number of sputum samples collected	Number of samples positive for *M. bovis* by PCR
Abattoir workers	Data not available	141	16	4
Butchers	Data not available	317	29	0
Livestock farmers	Data not available	50	50	1
Veterinarians	25	3	3	0
Veterinary assistants	68	5	5	0

Table 2. Bivariate frequency analysis of various parameters of the abattoir workers

Parameter	Negative	Positive *n* (%)	Chi-square
Age (years)			NS
<20	8	0 (0)	
20–30	30	0 (0)	
31–40	45	1 (2)	
41–50	37	2 (5)	
>50	17	1 (6)	
Education			NS
Illiterate	37	2 (5)	
Middle	73	1 (1)	
Secondary	25	1 (4)	
Graduate	2	0 (0)	
Duration of work (years)			<0.05
<8	80	0 (0)	
8–15	37	2 (5)	
>15	20	2 (10)	
Type of work			NS
Live animals handling	17	2 (12)	
Skinning and slaughtering	79	2 (2)	
Cleaning	16	0 (0)	
Other	25	0 (0)	

NS, not significant ($P > 0.05$).

handling, 81 (57%) in slaughtering and skinning, 16 (11%) in cleaning and 25 (18%) in other activities like transportation and purchasing. None of the abattoir workers had undergone any sort of formal training related to their work. Only three abattoir workers stated that they had a health certificate declaring them free of zoonotic diseases. These three workers considered themselves at risk of acquiring disease while working in the abattoir. Due to this concern, they had annual checkups in a tertiary care hospital and obtained a health certificate for their personal satisfaction. Obtaining a health certificate is not mandatory and indicates their attitude toward zoonotic diseases and whether or not they consider themselves at risk.

The majority of workers (83%) stated that they had direct contact with blood and carcasses.

None of the abattoir workers used face masks, hair nets or gloves. About 40% (57) used gumboots and 21% (29) used aprons for their protection. According to the abattoir workers, 94% (133) took regular showers before starting work and all took showers after finishing during the summer, while during winter (62.2%), they took regular shower before starting and 80% after finishing work. Hot water and sanitizers were not available to abattoir workers for cleaning themselves. Abattoir workers stated that veterinary doctors performed anti-mortem inspections of animals on a regular basis and that to their knowledge no animal had been culled during anti-mortem inspection as a result of being infected with bTB as animals were not screened for bTB. The abattoir workers also stated that meat inspections were not performed after slaughter and to their knowledge no animal had been condemned partly or as a whole due to the presence of tuberculous lesions. About 96% (136) of workers stated that they had at some point heard about TB. Regarding its transmission, 29, 62 and 45 stated correctly that TB could be transmitted from animals to humans through raw milk consumption, raw meat consumption and aerosols, respectively. We found that 27% of workers had no knowledge of TB transmission from animals to humans. The level of knowledge regarding signs and symptoms of TB was extremely poor (Table 3). The National Tuberculosis Control Programme of Pakistan states that a combination of 'chronic cough lasting for >3 weeks, fever and weight loss' are the main symptoms of TB. Only two of respondents (1%) knew the combination of these three signs. The majority of abattoir workers (89%) showed an interest in getting training on zoonotic diseases.

Of all 317 butchers working in 100 randomly selected meat retail shops, 29 were suspected of having TB and sputum samples were obtained from them. None were confirmed positive for *M. bovis* by PCR analysis. None of the butchers had any form of formal training related to their work and none had a health certificate. All of them washed their hands with soap before and after work. Among the butchers, 83% responded that during summer, they showered after finishing work while only 36% did so during winter. About 28% (90) stated that they removed all visible lesions from carcasses and offal while the remaining 72% stated that they removed visible lesions if customers insisted on this. When asked what they would do if a carcass was confirmed to be infected with bTB, all the butchers stated that they would only remove the lesions of bTB and would not condemn the whole carcass. The majority (92%) stated that they had direct contact with blood and carcasses. Aprons were used by 24% (75) of butchers, while none used gloves, facemasks or hair nets. All the participants believed that cattle and buffalo can be infected with bTB and more than half (60%) knew that bTB transmission from animals to humans can occur through consumption of raw meat, while 49% knew about aerosol transmission and 45% knew about transmission through faeces and urine. None knew the combination of three main symptoms of TB.

Of 50 sputum samples collected from livestock farmers, only one was positive for *M. bovis* by PCR. Age and education of the livestock farmers were not associated with *M. bovis* infection. The majority (44%) were >50 years of age and 46% had secondary education. None of the farmers had any formal training. The majority (62%) drank boiled milk daily, 16% drank boiled milk occasionally while 8% consumed raw milk occasionally. Only 10% (5) of farmers used gum boots and one used gloves during animal handling and cleaning of

Table 3. Distribution of signs and symptoms of TB known to the abattoir workers, butchers and livestock farmers

Signs and symptoms	Group	Yes, *n* (%)	No, *n* (%)
Chronic cough lasting for >3 weeks	Abattoir workers	34 (24)	107 (76)
	Butchers	85 (27)	232 (73)
	Livestock farmers	11 (22)	39 (78)
Pain in the chest	Abattoir workers	31 (22)	110 (78)
	Butchers	71 (22)	246 (78)
	Livestock farmers	10 (20)	40 (80)
Coughing up blood or sputum	Abattoir workers	32 (23)	109 (77)
	Butchers	79 (25)	238 (75)
	Livestock farmers	12 (24)	38 (76)
Weakness or fatigue	Abattoir workers	91 (64)	50 (36)
	Butchers	201 (63)	116 (37)
	Livestock farmers	30 (60)	20 (40)
Weight loss	Abattoir workers	101 (72)	40 (28)
	Butchers	225 (71)	92 (29)
	Livestock farmers	33 (66)	17 (34)
No appetite	Abattoir workers	121 (86)	20 (14)
	Butchers	252 (80)	65 (20)
	Livestock farmers	39 (78)	11 (22)
Chills	Abattoir workers	31 (22)	110 (78)
	Butchers	75 (24)	242 (76)
	Livestock farmers	21 (42)	29 (58)
Fever	Abattoir workers	136 (96)	5 (4)
	Butchers	273 (86)	44 (14)
	Livestock farmers	42 (84)	8 (16)
Sweating at night	Abattoir workers	21 (15)	120 (85)
	Butchers	43 (14)	274 (86)
	Livestock farmers	5 (10)	45 (90)
Vomiting	Abattoir workers	51 (36)	90 (64)
	Butchers	124 (39)	193 (61)
	Livestock farmers	39 (78)	11 (22)
Diarrhoea	Abattoir workers	67 (48)	74 (52)
	Butchers	156 (49)	161 (51)
	Livestock farmers	34 (68)	16 (32)

the animal shed. Soap was used by only 62% of farmers. All the farmers had heard about TB. The majority (88%) believed that TB in humans can be cured completely by adhering to treatment. None knew the combination of three main symptoms of TB. None of the farmers had ever screened their animals for bTB. A small number (4%) correctly stated that bTB is not treatable in animals. When asked what would they do if an animal were diagnosed with bTB, the majority (72%) responded that they would treat it as they did not know that bTB treatment is not recommended. About 26% (13) stated that they would sell the animal in the live animal market. None stated that they would cull and bury the diseased animal. The majority of the farmers (86%) showed an interest in training about zoonotic diseases.

Sputum samples from three veterinarians and five veterinary assistants were collected and all were negative on PCR analysis. The veterinarians' knowledge of TB signs and symptoms, transmission and the causative agent was excellent but veterinary assistants had poor knowledge.

Discussion

Our study found that abattoir workers were more commonly affected by zoonotic TB than the other professions studied. bTB is endemic in the area and abattoir workers were exposed both to live animals during preslaughter handling and to carcasses after slaughtering, Furthermore, they handled large numbers of animals daily and personal protective equipment was not available. Various studies conducted in bTB endemic countries have reported an increased risk of acquiring *M. bovis* infection among abattoir workers [18–21]. Abattoir workers involved in slaughtering or performing necropsies on infected animals are likely to be at a higher risk of acquiring zoonotic TB than livestock handlers, because of the potential for aerosol dispersion of bacilli [6]. Many abattoir workers were unaware of the zoonotic nature of bTB, its public health implications and its mode of transmission. None of them had undergone any sort of formal training on bTB. Around the world in developing countries, most zoonotic TB cases have occurred in abattoir

workers, veterinarians, dairy farm workers, hunters and zoo workers [6,9,18,20,22]. The level of knowledge of abattoir workers, butchers and livestock farmers regarding signs and symptoms of TB and its means of transmission was poor, suggesting the need for training of high-risk occupations as a high priority [9,23].

This is consistent with findings from the few studies that have assessed knowledge and attitudes among high-risk groups in some African countries. One study on the knowledge and prevailing practices of livestock handlers and animal health workers in Tanzania concluded that, sparse awareness and lack of knowledge of zoonoses, combined with food consumption habits and poor animal husbandry practices are likely to expose respondents to an increased risk of contracting zoonotic diseases [24]. Another study reported poor knowledge of TB in 75% of the respondents from rural areas of Tanzania and considered 40% of the respondents practices to be 'high risk' for exposure to bTB [25]. Lack of accurate knowledge on transmission of zoonotic diseases and the prevalence of high risk behaviour, such as unsafe slaughtering practices and consumption of raw animal products, have also been documented in Ethiopia [26]. Studies on knowledge and practices regarding bTB among livestock handlers, abattoir workers and butchers in Pakistan are scarce. Among the occupationally exposed groups included in the study, veterinarians and veterinary assistants had formal education related to their job. The standard of knowledge of veterinarians regarding TB may be linked to their formal education. However, veterinary assistants had poor knowledge, indicating a poor standard of work-related education and a need to improve their training with more emphasis on zoonotic diseases and handling of diseased animals.

Our study does have some limitations, in that we only studied the prevalence of active pulmonary TB in occupationally exposed risk groups. There could be cases of latent TB and extra-pulmonary TB in these groups which were not detected as our diagnosis was based on analysis of sputum samples.

Bovine and human TB affect all sectors of the community, but poor people are most vulnerable [27]. Urban and peri-urban livestock farming is growing fast but most livestock professionals and handlers in Peshawar are small-scale farmers, herders and unemployed people who are also poor and uneducated. Supporting education, animal husbandry training, capacity development and constant reassessment of animal handlers and related professionals are therefore critical to good health (both human and animal) and improving animal productivity in Peshawar. The findings of this study indicate the need for a bTB control programme to improve public health. The study also highlighted the need for improved awareness of zoonotic TB among livestock workers, abattoir workers and butchers, along with formal work-related training. Our findings also suggest the need for further in-depth investigations of the disease in the Peshawar district.

Key points

- Occupational groups exposed to bovine tuberculosis in Peshawar, Pakistan, do not employ appropriate safe working methods and personal protective equipment, which may put them at risk of acquiring zoonotic tuberculosis.
- Duration of work as an abattoir worker was associated with the prevalence of zoonotic tuberculosis in this occupational group.
- The abattoir workers, butchers, livestock farmers and veterinary assistants interviewed in this study had poor knowledge of tuberculosis despite their increased occupational risk of exposure.

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Conflicts of interest

None declared.

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