Journal of Microbial Pathogenesis

ullah et al., J Microb Path 2018, 2:1

Research Article Open Access

An Emerging Zoonosis of Bovine Tuberculosis-A Neglected Zoonotic Disease (NZD) in Work-Related Occupational Groups in Pakistan

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Received July 14, 2018; **Accepted** July 30, 2018; **Published** August 20, 2018

Abstract

Background: Tuberculosis (TB) is potentially serious infectious disease caused by the bacterium *Mycobacterium tuberculosis* (MTB). *Bovine tuberculosis* (BTB) is found one of the neglected zoonotic disease (NZD) caused by *Mycobacterium bovis* has an economic importance and public health significance and appear as a life threatening infection at the human-animal-ecosystem interface in low-resource settings and less developed countries across the world. Employees in different categories of work may be deliberately or unintentionally exposed to biological agents. Lack of information leads to spread of an infection. Globally, insufficient information is available on human TB caused by *Mycobacterium bovis* in emerging states.

Aims: To specify the incidence of pulmonary tuberculosis due to *M. bovis* in specific work-related occupational groups such as livestock farm workers, abattoir workers, butchers, veterinarians and veterinary assistants and also to document their awareness, level of knowledge and practices concerning bovine tuberculosis.

Materials and Methods: A study was conducted on livestock farm workers, abattoir workers, butchers, veterinarians and veterinary assistants. Data was obtained on socio-demographic conditions, awareness, level of knowledge and practices about tuberculosis. The sputum samples were also collected from those individuals who were suffering from cough and finally confirmed by PCR. Data was finally analysed statistically using chi squared test.

Results: A total of 390 sputum samples belonging to 800 study participants were collected which consisted of 100 TB patients admitted in different human hospitals in the study area, 200 livestock farm workers, 174 abattoir workers, 294 butchers, 10 veterinarians and 22 veterinary assistants. As a result, 2 out of 100 TB patients (2/100), 3 out of 200 livestock farm workers (3/200) and 3 out of 23 abattoir workers (3/23) were found positive for the presence of *M. bovis* through PCR technique. A significant association ($p<0.05$) was found between livestock farm workers and abattoir workers with the prevalence of zoonotic tuberculosis.

The level of knowledge and awareness of livestock farmers, abattoir workers, butchers and veterinary assistants concerning the zoonotic spread of BTB and the signs & symptoms of human TB was very miserable. Majority of the manual workers were unaware about the principles of biosafety and biosecurity and did not use personal protective equipment (PPE) and were considered high risk occupational groups of gaining zoonotic TB.

Conclusions: *Bovine tuberculosis* is a major public health problem in especially work-related occupational groups in district Peshawar, the capital city of Khyber Pakhthunkhwa, Pakistan. The surveillance, prevention and control programs of this NZD in the province Khyber Pakhthunkhwa, Pakistan is necessary because of the continual unrestricted movement of the animals which might result in an increased spread of BTB to humans.

Keywords: BTB; NZD; *Mycobacterium bovis*; work-related occupational groups; PCR; Peshawar; KPK

Introduction

Tuberculosis (TB) remains a major cause of high mortality worldwide and a serious public health threat [1]. More than 95% of TB deaths occur in the developing world [2]. Mycobacterium tuberculosis is known to account for most cases of human TB; however, the proportion due to *M. bovis* is unknown [3]. *Bovine tuberculosis* (BTB) is mainly caused by *M. bovis* ssp. bovis and to a lesser extent- *M. bovis* ssp. caprae [4,5]. *M. tuberculosis*, *M. africanum*, *M. pinnipedii*, *M. canetti*, *M. microti* along with vaccination strain Bacillus Calmette-Guerin form the Mycobacterium tuberculosis complex (MTC). Like Corynbacteriaceae, Tsukumurellaceae, and Nocardiaceae, Mycobaterium spp. is Actinomycetales [6-8]. Cattle signify the chief reservoir of BTB however there is a rare human-human transmission [9]. Incidences which are reported under WHO: 0.002 % (2/100,000) in African region, 0.007 % (7/100,000) in American region, 0.03 % (30/100,000) in European region [10]. BTB is one of the neglected zoonotic diseases (NZD) at the human-animal-ecosystem interface and has been reported to cause disease in humans in widespread regions across the world [11]. A survey was conducted by the World Health Organization in 2016, where in approximately 1.3 million deaths were reported among HIV-negative individuals (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive individuals. Similarly an estimated 10.4 million people fell ill with TB in 2016 in with 90% were adults, 65% were male, 10% were people living with HIV (74% in Africa) and 56% were in five countries (listed in descending order of their number of incident cases) i.e; India, Indonesia, China, the Philippines and Pakistan [12]. Globally, Pakistan is estimated to

have the 4th highest prevalence of multidrug-resistance TB (MDR-TB) [12]. An increase in AIDS and drug resistance has been result in high incidence of human tuberculosis (TB) [13]. The problem has been aggravated by an increase in the incidence of human immunodeficiency virus (HIV) and by the emergence of multidrug resistance [13]. Human immunodeficiency virus (HIV) plays a significant role in development and succession of active TB among all those individuals infected by HIV and TB [14]. Mycobacterium tuberculosis is the most common cause of TB in humans but humans are also infected by *Mycobacterium bovis* that causes *bovine tuberculosis* (BTB) [15]. The diseases that are transmitted from animals (vertebrates) to humans are called zoonoses. In human, majority of newly emerging infections are of zoonotic origin [16]. Most zoonoses occur during preparation of infected meat and ingestion or close contact to animals during hunting, herding or slaughtering animals [17]. Transmission of *Mycobacterium bovis* occur either inhalation or oral route [18]. All the developed countries have been succeeded in reducing the transmission of TB in humans with the help of proper pasteurization of dairy products and proper control of BTB infection in bovines [19,20]. Although TB in human is important zoonotic disease reported globally where BTB is endemic and improper pasteurization of milk and other dairy products [21]. All the developed countries are poorly documented about data regarding human TB due to *M. bovis* (zoonotic TB) due to poor diagnostic facilities such as sputum cytology only. Pakistan has been ranked fifth country among high burden countries (HBC) globally where 193 million total populations has been reported [12].

The World Health Organization (WHO) has suggested the proper collection of information regarding zoonotic diseases in all developing countries [22]. Here in Pakistan, we have no regular system for screening of animals for presence of BTB using tests tuberculin dermal test. Different researchers reported dissimilar prevalence in different areas of research institutions such 6%, 8% and 2.5% & 10% in Peshawar, Lahore and different livestock farms in Punjab area respectively [23-26]. In Peshawar, a study was conducted at a slaughterhouse to record BTB in bovines and as result 13% and 14% prevalence was detected in cattle and buffaloes respectively [27].

In the present study prevalence of BTB in bovines and the zoonotic aspect was studied because limited data was available in livestock handlers, abattoir workers, veterinarians and butchers. Unluckily, here in Pakistan we have no known data to operate BTB control program successfully and aware our public against prevention strategies [1]. Therefore this study was designed and conducted to find out the prevalence and associated risk factors responsible for an outbreak of pulmonary TB (*M. bovis*) in livestock farm workers, slaughter house workers, animal handlers, butchers, veterinary assistants and veterinarians. Furthermore, the awareness and different practices of these professionals regarding TB was also studied.

Materials and Methods

Ethical approval

This research study was approved by the Ethical Review Committee, The University of Agriculture Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan.

Sampling and methodology

The present research was carried out in district Peshawar where samples were collected and analyzed from January 2017 to December 2017. The district is the capital city of Khyber Pakhtunkhwa (KPK) province with approximately 3.52 million human populations and is close to Afghanistan border. There are two large abattoirs one in Town-1 on ring road and other in Town-II on Landi sarak and is run by provincial government. There are with five other abattoirs with are administered privately. The animal health services are provided in the study area either through veterinary hospitals or veterinary dispensaries administered by provincial government/privately. The participants of the study were abattoir workers from the two large abattoirs run by provincial government, livestock farm workers with signs of cough, veterinary assistants and veterinarians. A total of 355 abattoir workers involved in routine work at both government run abattoirs. Slaughtering of different breeds is carried out in three phases. In phase-I, large ruminants with < 2years were slaughtered from 08:00am to 04:00pm. In phase-II, large ruminants (Cattle & Buffalo) with >2years were slaughtered from 04:00pm to 01:00am while in phase-III, small ruminants (Sheep & Goat) were slaughtered from 01:00am to 08:00am. The administration of the slaughter house adopt tough selection criteria for both large and small ruminants whether the animals came for slaughtering fulfil the basic requirement for the same or not. Animals are rejected on the basis of body weight and physical condition. Underweight (i.e.; large ruminants with < 60kg and small ruminants with < 11kg) and put-down animals which cannot stand by them are categorized as rejected animals. Even animals which are already slaughtered outside the abattoir are not accepted. The purpose of the study was explained to all the abattoir workers and those who decided to participate were included in the study. A random sampling technique was followed and included 200 livestock farm workers who were exhibited clinical signs of cough up sputum and 100 TB patients who were admitted in different hospitals in the study area. Different dairy farms and farmers community associations were visited and history, physical examination and family information were recorded. Similarly, history of chronic cough and sputum samples were collected from veterinarians and veterinary assistants working in the study area.

The author (Asad ullah) used a predesigned feedback form and carried out the exchange of ideas in Pushto (local dialectal). The interview was focused on tuberculosis and the knowledge, awareness, practices and socio-demographic conditions of the participants with respect to tuberculosis. Distinct feedback form was designed for abattoir workers, slaughterers, veterinarians, veterinary assistants and livestock farm workers.

DNA extraction and PCR

The processing of sputum samples and isolation of DNA was carried out using Genolyse ® DNA kit in the biosafety level III at Provincial Tuberculosis Reference Laboratory, Peshawar. The PCR protocol as adopted by I. Khattak et al., 2016 was followed with some modification for molecular detection of *Mycobacterium bovis* in sputum samples. The detection of *M. bovis* was carried out by amplification of 500 bp PCR product with specie-specific primers as JB primers (JB21 as Forward: 5'-TCGTCCGCTGATGCAAGTGC-3' and JB22 as Reverse: 5'-CGTCCGCTGACCTCAAGAAG-3').

The PCR was optimized and carried out in a BIORAD T100TM, Biorad® thermal cycler with 25 µl reaction volumes for each sample having 1.75 µl of each forward (F) and reverse (R) primer, 10 µl master mix, 2.5 µl of DNA sample and 9 µl DNAas-free deionized water. The protocol for amplification involved denaturation, annealing, and extension steps at 950C for 30sec, 550C for 30sec and 720C for 1minute respectively. All the samples were subjected to 35 cycles before a final 5 minutes extension at 720C and 500 bp amplified products were obtained over 35 cycle. The PCR amplified products were visualized with UV illumination (Figure 1).

Gel electrophoresis

A 100ml 10X TAE buffer was taken in a 100ml flask and 1.5 gram agarose was dissolved by keeping the flask in microwave oven for 1 minute and 30sec. Then, 4µl SYBR® Soft gel stain by Thromo-Fisher Scientific (which help in visualization of amplified PCR product) was added to the flask before pouring into gel tray. The gel tray was then transferred to electrophoresis tray filled with 10X TAE buffer. All the PCR-products and 6µl DNA-ladder (1000bp) was then loaded into gel-wells using micropipettes and power supply (120volte, 500mAmp for 35 minutes) was applied. Finally, the gel was observed in gel-documentation system and was photographed (Figure 2).

Statistical analysis

The level of significance was calculated by applying Chi squared test with 95% confidence intervals after the data was processed and analysed with SPSS (Version 16.0). The percent prevalence of the disease was presented in the form of frequencies and percentages.

Results

A total of 390 sputum samples belonging to 800 study participants were collected which consisted of 100 TB patients admitted in different human hospitals in the study area, 200 livestock farm workers, 174 abattoir workers, 294 butchers, 10 veterinarians and 22 veterinary assistants. The collected sputum samples were processed and analysed for the detection of *M. bovis* through Ziehl-Neelsen staining, culture and polymerase chain reaction (PCR). Out of 100 sputum samples collected from TB patients, 100% were found positive for acid fast bacteria through Ziehl-Neelsen staining (Figure 3) while 2% were positive for *M. bovis* through culture and PCR each (Table 1, Figure 1). Similarly, a total of 200 livestock farm workers were interviewed and sputum samples were collected from them and were analysed for the presence of *M.bovis*. 2 samples were found positive through Ziehl-Neelsen staining (Figure 4) while 3 samples were found positive through culture and PCR each for the presence of *M. bovis* (Table 1, Figure 1). Type of work, duration of work (years), age (years) and education of the livestock farm workers were significantly associated ($p<0.05$) with occurrence of zoonotic TB (Table 2). Among the farm workers, 10 (5%) were involved in watering and feeding while 12 (6%) farm workers were involved in milking, 92 (46%) in cleaning the overall farm, 22 (11%) performed all tasks and 64 (32%) in other farm activities i.e; procuring and carriage. None of the farm workers had undergone any sort of formal training related to their job at livestock farm. Similarly, the farm workers working for >15 years at the farm were found more positive for TB as compared to the workers working for < 15 years at the livestock farm. During the research, the disease found significantly associated with old age of the farm workers. The livestock farm workers with age >45 years were found more positive for TB as compared to young workers. Likewise, the disease was not detected in the educated farm workers and was found in the illiterate group or those who got primary education only (Table 2).

During the investigation, the entire livestock farm workers included in this study was male and interviewed. 39 % (78) were found with age above 45years & 41% (82) with education at secondary level. No single farmer was found with any proper work related training. Similarly, 57% (114) of the livestock farm workers consumed boiled milk regularly, 12 %(24) consumed boiled milk occasionally whereas 7% (14) used whole milk rarely. Simply 9% (18) of livestock farm workers used gumboots and no one used gloves in the course of animal management and cleaning of the livestock shed. Only 59% (118) of the farm workers used soap for hand and body washing. 100% of the farm

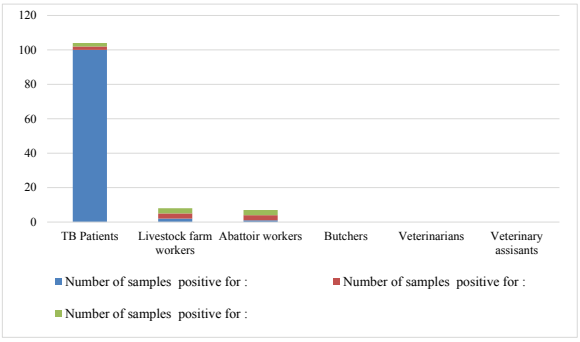

Figure 1: Graph showing the overall results of Ziehl-Neelsen staining, Culture and PCR of the Occupational groups in the study area.

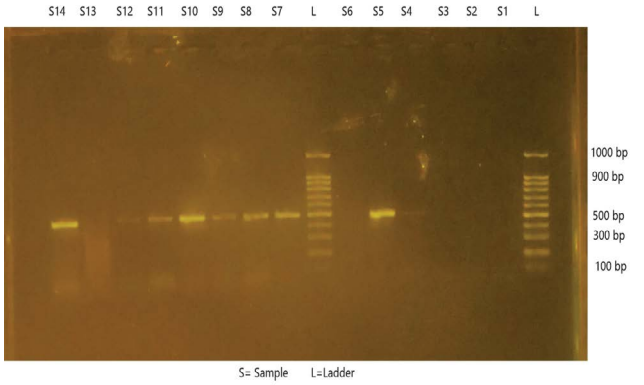

Figure 2: PCR amplicon showing specie-specific 500bp DNA of *Mycobacterium bovis* (S5, 7-12 and S14). S1-4, 6 & 13 are negative samples.

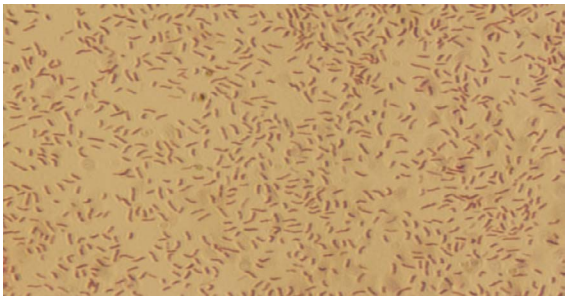

Figure 3: Ziehl-Neelsen staining of sputum samples of TB patients showing acid-fast bacilli.

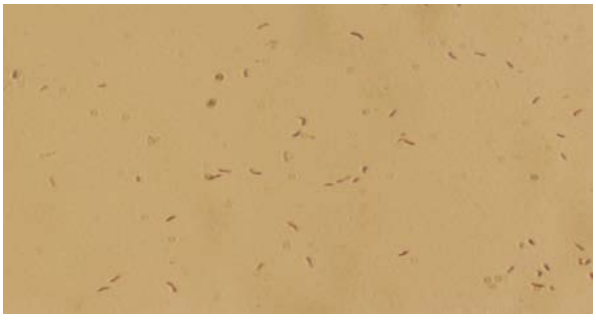

Figure 4: Ziehl-Neelsen staining of sputum samples of livestock farm workers showing acid-fast bacilli

Group	Total number in study area	Number interviewed	Number of sputum samples collected	Number of samples positive by Ziehl-Neelsen for Acid Fast bacteria	Number of samples positive for *M. bovis* by culture	Number of samples positive for *M. bovis* by PCR
TB Patients	Data not available	100	100	100	2	2
Livestock farm workers	Data not available	200	200	2	3	3
Abattoir workers	Data not available	174	23	1	3	3
Butchers	Data not available	294	35	0	0	0
Veterinarians	33	10	10	0	0	0
Veterinary assistants	76	22	22	0	0	0

Table 1: Sample distribution and PCR results in the study area.

Parameter	Negative	Positive N(%)	Chi-square
Type of work at livestock farm			
Watering & Feeding	9	1(10.00)	*p= 0.014*
Milking	11	1(8.33)	
Cleaning	92	0(0.0)	
All tasks	21	1 (4.55)	
Others	64	0(0.0)	
Duration of work (years) at livestock farm			
< 6	137	0(0.0)	*p=0.036*
7–15	21	1(4.55)	
>15	39	2(4.88)	
Age of farm worker (years)			
upto 18	40	0(0.0)	*p= 0.015*
19–25	54	0(0.0)	
26–35	66	0(0.0)	
36–45	26	2(7.14)	
> 45	11	1(8.33)	
Education of farm worker			
Illiterate	21	2(8.70)	*p=0.005*
Primary	10	1(9.09)	
Secondary	78	0(0.0)	
Higher Secondary	39	0(0.0)	
Graduate	49	0(0.0)	

Table 2: Bivariate frequency analysis of various parameters of the livestock farm workers.

workers had heard about TB. 84% (168) of the livestock farm workers assumed that human TB can be treated completely by following the treatment. None of the farm worker was aware of the amalgamation of 3 key symptoms of TB. None of the farm worker had ever screened their farm for BTB. Only 3% (6) properly indicated that BTB is not curable in animals. As the farmers was not aware that globally the BTB treatment is not recommended so when they were asked what they would do if an animal was found affected with BTB. Only 68% (136) of the respondents specified that they would do medication for the same. 22% (44) identified that they would sell the TB affected animal in the market. None of the farmer indicated that they would cull and bury the TB affected animal. Almost 81% (162) of the farm workers revealed a curio in education and training about infectious and zoonotic animal diseases.

A total 174 abattoir workers were interviewed where 23 had been suffering from chronic cough. 23 Sputum samples were collected for the presence of biological agent i.e; *M. bovis*. Of these, 1 sample was found positive through Ziehl-Neelsen staining (Figure 5) while 3 samples were found positive for the presence of *M. bovis* through culture and PCR each (Table 1, Figure 1). All the abattoir workers included in this study were male and the reply rate of the slaughter house labour was around 93% (174/187). Type of work, duration of work (years), age (years) and education of the abattoir worker was significantly associated ($p<0.05$) with incidence of zoonotic TB. Those who had worked for more than 10 years in the abattoir were more affected by zoonotic TB as compared to those who worked for a short time. All the selected slaughter house workers were interviewed and it was documented that 77% (134) were having direct interaction with animal blood and dead bodies. Personal protective equipment (PPE) like gloves, masks and hair nets were used by only 2 %(6) of the abattoir workers while gumboots and aprons were used by 35% (61) and 19% (33) respectively. Similarly, before going to start work, 88% (153) of abattoir workers took baths. In winter 59% (103) took steady bath before going to start work and 73% (127) took bath when finished their job while during summer, all workers at slaughter house took baths when finished their job. Other facilities like warm water, antiseptics and disinfectants were not offered at slaughter house to workers for their washing and for the equipment they used during work at abattoir. It was stated by the abattoir workers that on job veterinarian do anti-mortem inspections normally. As animals were not screened for BTB hence, no animal had been rejected due to the presence of BTB infection, during the inspection. Further, after butchery, due to the presence of tuberculous lesions, no animal had put away neither partly nor as a whole so far. Similarly, knowledge about BTB and its transmission to human was also poor. It was documented that 23% (40) of abattoir workers had no knowledge of TB and its zoonotic transmission. Only 86% (150) of slaughter house workers indicated that they had heard about TB while 39% (68) workers

Signs and symptoms	Group	Yes, n (%)	No, n (%)
Coughing up blood or sputum	TB patients	100 (100)	0 (0)
	Livestock workers	48 (24)	152 (76)
	Abattoir workers	35 (20)	139 (80)
	Butchers	76 (26)	218 (74)
Chronic cough lasting for >3 weeks	TB patients	100 (100)	0 (0)
	Livestock workers	40 (20)	160 (80)
	Abattoir workers	33 (19)	141 (81)
	Butchers	74 (25)	220 (75)
Pain in the chest	TB patients	99 (99)	1 (1)
	Livestock workers	44 (22)	156 (78)
	Abattoir workers	26 (15)	148 (85)
	Butchers	71 (24)	223 (78)
Weight loss	TB patients	100 (100)	0 (0)
	Livestock workers	132 (66)	68(34)
	Abattoir workers	120 (69)	54 (31)
	Butchers	197 (67)	97 (33)
Weakness or fatigue	TB patients	100 (100)	0 (0)
	Livestock workers	100 (50)	100 (50)
	Abattoir workers	90 (52)	84 (48)
	Butchers	173 (59)	121 (41)
Annorexia	TB patients	99 (99)	1 (1)
	Livestock workers	160 (80)	40 (20)
	Abattoir workers	146 (84)	28 (16)
	Butchers	229 (78)	65 (22)
Fever	TB patients	99 (99)	1 (1)
	Livestock workers	156 (78)	44 (22)
	Abattoir workers	136 (89)	19 (11)
	Butchers	235 (80)	59 (20)
Chills	TB patients	99 (99)	1 (1)
	Livestock workers	80 (40)	120 (60)
	Abattoir workers	42 (24)	132 (76)
	Butchers	82 (28)	212 (72)
Sweating at night	TB patients	99 (99)	1 (1)
	Livestock workers	32 (16)	168 (84)
	Abattoir workers	30 (17)	144 (83)
	Butchers	47 (16)	247 (84)
Diarrhoea	TB patients	36 (36)	64 (64)
	Livestock workers	140 (70)	60 (30)
	Abattoir workers	92 (53)	82 (47)
	Butchers	153 (52)	141 (48)
Vomiting	TB patients	41 (41)	59 (59)
	Livestock workers	160 (80)	40 (20)
	Abattoir workers	66 (38)	108 (62)
	Butchers	126 (43)	168 (57)

Table 3: Distribution of signs and symptoms of bovine tuberculosis known to the livestock workers in the study area.

indicated the transmission of TB occur from animals to human via the consumption of raw milk. Likewise, 54% (94) declared that the transmission occurs via the consumption of raw meat and 46% (80) declared aerosols route for the transmission of TB from animals to humans. It was found that the majority of abattoir workers have no knowledge about BTB and its zoonosis.

Only 7% (14) livestock farm workers and 3% (5) abattoir workers indicated that they had a health certificate declaring them free of zoonotic diseases. These 14 livestock farm workers and 5 abattoir workers considered themselves at risk of getting diseases while working with animals at livestock farm and in abattoir respectively. These occupationally exposed workers conducted complete body check-up once per annum in a tertiary care hospital and obtained health certificate for their own satisfaction. Although, proper annual body check-up and getting health certificate is not compulsory for the occupationally exposed workers in the country. However, the attitude of the livestock farm and abattoir workers weather or not they consider themselves at risk of infection of zoonotic diseases are reflected by acquiring a proper health certificate from the office of the district TB control officer (DTCO). The level of knowledge of the workers about signs and symptoms of TB was very pitiable (Table 3). The National Tuberculosis Control Program (NTCP), Pakistan have declared the combination three signs i.e. "chronic cough lasting for more than 3

Figure 5: Ziehl-Neelsen staining of sputum samples of abattoir workers showing acid-fast bacilli.

weeks, weight loss and fever" as the main symptoms of TB. Hence, in this study, only 3 (2%) of the abattoir workers knew the combination of these three signs. Similarly, 83% (144) of the slaughter house workers were demanding training on zoonotic diseases.

A total of 100 meat retail shops were selected randomly in the study area. Out of 294 butchers working in these meat retail shops, 35 sputum samples were collected from those butchers who were doubted of having TB. All the collected sputum samples were processed and analysed through Ziehl-Neelsen staining, culture and final confirmation was made through PCR. All the samples were negative for the presence of *M. bovis*. All the butchers answered that they washed their hands before starting and after finishing the work regularly. Only 29% (85) butchers replied that they acquired bath after finishing the work during winter while 86% (253) did so during summer. 33% (97) butchers identified that they removed all visible lesions from carcasses while 67% (197) indicated that they removed visible lesions on customer's desire. All the butchers quantified that they would remove only the BTB infected area along with some normal tissues and would not discard the whole carcass, when they were asked if a carcass was confirmed to be infected with BTB. Furthermore, 89% (262) butchers indicated that they had direct contact with blood and dead bodies of animals. 19% (56) of the butchers used aprons while gloves, facemasks or hair nets were used by only 2% (6). No butcher had a health certificate and no one had any form of formal training related to their work. During the investigation, all the butchers specified that buffalo and cattle can be infected with TB. Similarly, 58% (171) identified that transmission of BTB from animals to humans occur via consumption of raw meat, 43% (126) butchers stated zoonotic transmission of BTB occur via aerosol route while 39% (115) stated that the zoonosis of BTB occur through faeces and urine. So far the awareness about three standard signs of TB according to NRCP of Pakistan, no single butcher knew the combination of three signs which are "chronic cough lasting for more than 3 weeks, weight loss and fever" as the main symptoms of TB.

Similarly, the sputum samples were collected from 10 veterinarians and 22 veterinary assistants working in the study area. All the collected samples were processed and analysed through Ziehl-Neelsen staining, culture and final confirmation was made through PCR. All the samples were negative for the presence of *M. bovis*. The veterinarians and the veterinary assistants were also interrogated about the TB, the etiological agent, the signs & symptoms of TB and the zoonotic transmission of BTB. The knowledge of veterinarians was outstanding but veterinary assistants had poor understanding of the same.

Discussion

Our study found that as compared to other professions studied in this study, the livestock farm workers and slaughter house manual labourer were affected as a result of zoonotic tuberculosis more frequently. As *bovine tuberculosis* is prevalent in study area and the livestock farm workers during farm management were found unprotected from live farm animals. Similarly, abattoir labourers were also found uncover equally from live animals (in the course of pre-slaughtering management) and carcasses (during and after slaughtering). Moreover, livestock farm workers and the abattoir workers, daily manage variety of large number of animals and personal protective equipment (PPE) was available to none of them. Several research studies were conducted in many endemic countries of BTB and have reported that abattoir workers were at increased risk of getting *M. bovis* infection [28-31]. As compared to livestock farm workers, the slaughter house workers were found at a much greater risk of contracting zoonotic TB due to the aerosol dispersion of bacilli as they were involved in carrying out necropsies and slaughtering the animals having direct contact with blood and carcasses of infected animals [18].

Majority of the slaughter house workers were unacquainted about BTB, its way of transmission, its zoonosis and public health significance. No single slaughter house worker had got any formal training with respect to BTB. Globally in unindustrialized republics, maximum cases of zoonotic TB have been reported in livestock farm labours, abattoir staffs, zoo workers, predators and veterinarians [18,21,28,30,32]. The level of knowledge of livestock farm workers, abattoir workers and butchers about signs and indications of TB and the ways of its spreading was negligible which on priority basis signifying the prerequisite for proper and specific job related education and training of high risk groups [21,33]. Amongst high risk crowds, the awareness and approaches were evaluated and were found in line with the results of investigation carried out in some African states. Similarly, a study was conducted in the republic of Tanzania at countryside regions which revealed 40% respondent's animal husbandry practices are to be high risk on behalf of contracting BTB and 75% of the respondents were reported with underprivileged awareness of TB [34]. A similar study was conducted in Tanzania on awareness and usual farm workers practices and livestock healthcare workers. The study determined an increased risk to expose the respondents of contracting zoonotic diseases are due to certain factor like; lake of specific and job related trainings, lake of PPE's, un-hygiene food intake habits, poor livestock rearing practices and lake of awareness about zoonosis of certain chronic diseases [35]. Similarly, Lack of awareness on spreading of zoonotic infections and occurrence of high risk behaviour, for example consumption of raw animal products and unsafe slaughtering practices, has also been recognized in Ethiopia [36]. In Pakistan, the studies among abattoir workers, livestock farm workers and butchers the awareness and usual practices about the zoonotic BTB are very rare. In this study, the veterinarians and veterinary assistants had proper schooling amongst the occupationally unprotected groups related to their occupation. The standard of veterinarian's awareness about BTB is associated to their proper education in veterinary medicine while veterinary assistants had poor understanding and awareness about BTB demonstrating a reduced standard of education related to their job and demanding to improve their knowledge by giving them job related different types of trainings with more importance on transmission of zoonotic diseases, personal protective measures and management of sick animals. In our study, the results are based on the analysis of sputum samples, the extra-pulmonary TB and the latent TB could be the cases in the high risk occupationally exposed groups which were not detected. We have merely considered the occurrence of active pulmonary TB cases in these high risk professions. Although, all segments of the public are affected by BTB and human TB but poor community are most susceptible [37-40]. In Peshawar, livestock farming is growing fast in and around the

city. Majority of the livestock farmers have countable animals at their farm. However, maximum livestock farmers, livestock farm workers and professionals are poor and lacking basic knowledge related to their job. In this connection, it is serious to improve the capacity building by giving proper livestock farming training, supporting the education and perpetual re-evaluation of the livestock workers and allied professionals for the welfare and good health of both animal and human hence improving animal productivity in Peshawar.

Conclusions

Bovine tuberculosis is a major public health problem in especially work-related occupational groups in district Peshawar, the capital city of Khyber Pakhthunkhwa, Pakistan. In medical and veterinary laboratories, PCR could be applied to the clinical samples obtained from both human and animals for the detection of *M. bovis*. Though, a bit expensive, the PCR can be used as a routine diagnostic test to check the prevalence of *bovine tuberculosis* in cattle/buffaloe in Pakistan. On the basis of our findings, the surveillance, prevention and control programs of this NZD in the province Khyber Pakhthunkhwa, Pakistan is necessary because of the continual unrestricted movement of the animals which might result in an increased spread of BTB to humans.

Facts

- The occupational groups neither used personal protective equipment (PPE) nor adopted suitable safe working procedures, putting them at risk of getting zoonotic diseases like *bovine tuberculosis* in the capital city of Khyber Pakhtunkhwa, Pakistan.
- The type of work at livestock farm, duration of work, age and education of the livestock farm worker were linked with the prevalence of zoonotic TB in this occupationally exposed group.
- The livestock farm workers, abattoir workers, butchers and veterinary assistants who were interrogated in this study had less awareness and negligible knowledge about TB.

Recommendations

The findings of this research specify that improved risk assessment is required to identify different opportunities to stop work-related infectious diseases. The results also specify the intense need of political/government commitment to mitigate the impact of BTB on the citizen's health by implementing control programs to address this neglected zoonotic disease (NZD) at the human-animal-ecosystem interface. The investigation emphasized the want for better awareness of zoonotic TB amongst specific occupational group's i.e.; livestock farm workers, slaughter house labours and butchers along with formal education and job specific trainings. The results of this research study recommend the necessity for further and thorough research of this specific work-related infectious and zoonotic disease in and around the capital city-Peshawar of Khyber Pakhtunkhwa, Pakistan.

Acknowledgment

Financial support to conduct this study was provided by Higher Education Commission, Pakistan is gratefully acknowledged. The authors thank Mr. Sajid Ali (Technical Incharge/Molecular biologist) for providing technical support and culture facility at Provincial TB Reference Laboratory (BSL-III Lab), Peshawar, Khyber Pakhtunkhwa, Pakistan.

Conflict of interest statement

The authors have no conflict of interest to declare.

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