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PREVALENCE OF BOVINE TUBERCULOSIS IN SLAUGHTERING ANIMALS AT SELECTED MUNICIPAL SLAUGHTER HOUSES: ITS IMPACT ON PUBLIC HEALTH

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ABSTRACT

Bovine tuberculosis caused by *Mycobacterium bovis* is prevalent in animals in most of the developing countries, where surveillance and control activities are often inadequate or unavailable and pasteurization is rarely practiced. The objectives of the present study were to investigate the seroprevalence of bovine tuberculosis (bTB) in slaughtered cattle and buffaloes and to assess the health of abattoir workers for presence of *Mycobacterium bovis* causing as zoonotic disease. This cross-sectional study was conducted at two abattoirs of Karachi Metropolitan Corporation. Questionnaires were developed to collect data on: social demographics, medical information, work related exposure factors and preventive measures as well. The butchers and meat handlers were selected by simple random sampling. Cattle slaughtered at the abattoirs were also screened for bovine tuberculosis using lateral flow technique and Ziehl Neelsen test. Eight hundred serum and 300 lung tissue samples obtained from both abattoirs were screened for presence of *Mycobacterium tuberculosis.* Out of 800 serum samples 47 (5.8%) and 300 lung tissue samples, 38 (12.7%) were found positive by the rapid test lateral flow technique and Ziehl Neelsen stain, respectively. In this study 119 butchers and meat handlers were interviewed. The significant effect of knowledge and experience of both butchers and meat hadleers on potential zoonotic risk of disease was observed. Multivariate analysis of risk factor showed highly significant effect (P<0.001) of work experience >6 years as calculated by odd ratio of 3.7 (1.9-7.7), followed by potential risk associated with job was significant (P<0.02) having odd ratio of 2.7 (1.3-5.3). Meat consumed with diseases lesions had significant (P<0.05) effect with odd ratio of 2.5 (1.4-5.0). Eating or drinking at place of work showed no significant difference (P>0.05). A significant difference (P<0.01) was observed between male and female cattles with highly significant in female as compared to male cattles. No significant difference (P>0.05) was observed between positive sputum samples of butchers, meat handlers and abattoir workers stained with AFB staining method and by microscopic examination. It was concluded from the results of present study that there is a potential risk for zoonotic transmission of *Mycobacterium tuberculosis* from infected animal's slaugheterd at abbatoir to butchers and meat handlers. Knowledge and experience of work and refraining from use of infected meat may effectively reduce risk factors of transmission.

Keywords: abattoirs, bovine tuberculosis, butchers, TB prevalence

INTRODUCTION

Tuberculosis is a zoonotic disease caused by *Mycobacterium tuberculosis* in man and *M. bovis* in bovines (Smith *et al*., 2006; Pal, 2007; Malama *et al.,* 2013). Disease can be transmitted through aerosol of infectious organism expelled in the exhaled air, in sputum, urine, milk, feaces, vaginal and uterine discharges, and liberations from exposed peripheral lymph nodes of clinically or sub-clinically infected individuals (Leghari *et al.,* 2016). It adversely affects to reduce the production by about 10 to 25 percent; direct estimated losses in milk ranging from 10 to 18 percent and in meat about 15 percent (Radostits *et al*., 1994). In addition to economic losses, the tuberculosis also has an important public health importance (Müller *et al*., 2013). Presently, the human tuberculosis is getting increasing importance in rural areas of developing countries, where men and animals are sharing the same environment in houses and yard

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premises that results the increasing transmission of tuberculosis organisms to susceptible individuals (Shitaye *et al.,* 2007). In countries, where unpasteurized milk is used on routine basis, this disease is estimated as 10-15 percent in human population (Ashford *et al.,* 2001; Berg *et al*., 2015). In countries where routine post-mortem examination is obligatory, bovine tuberculosis has been observed as a major cause of carcasses condemnation.

Karachi is considered as the world's most populated city in Pakistan and people raise significant number of farm animals in its sub-urban areas. Regardless of the large number of livestock population, there is no data available on bTB, except for the fact that bTB emerges as a serious public health hazard and is also responsible for huge economic losses in dairy and meat industry. Therefore the present study was carried out to determine the prevalence of bovine tuberculosis in animals slaughtered at the abattoirs of Karachi Metropolitan Corporation (KMC) and to identify potential risk factors associated with zoonotic transmission of bTB from animals to human.

MATERIALS AND METHODS

Study area and population

The study was carried out in Karachi Metropolitan Corporation (KMC) abatttoirs where approximately, 1200 large animals (cows and buffaloes) were slaughtered every day.

Study design (for slaughtered animals, butchers and meat handlers)

Study population

Data collection and analysis

A questionnaire was developed to collect data from butchers and meat handlers of age of about 18 years and above. Animals available for slaughtering at abbatoir were physically examined for external body lesions before slaughter and their visceral organs were examined for presence of any pathological lesions. Prevalence of TB was calculated as the proportion of positive animals from the total number of animals visited (Thrusfield, 2005). Pre-slaughter examination data on sex, age, and body condition of animals were documented on a spreadsheet.

To evaluate the role of various possible risk factors involved in the incidence of TB in cattle, and its transmission to people who closely worked with those affected animals. A designed questionnaire form was distributed to 150 cattle owners, and slaughter house employees as to judge the insight of stakeholders on the incidence of bovine tuberculosis, socio economic status, livestock constraints, herd composition, awareness on the probable risk of zoonotic spread of bovine tuberculosis.

Animal samples and their analysis

Collection and analysis of blood samples for serological and molecular test

Blood samples (10 ml) were collected from each of 800 animals available for slaugheter. Blood was poured into plain vaccuntainer (BDH, UK) and kept for clotting. Then it was centrifuged at 10000 rpm for 10 minutes and serum was separated in 1.5 ml eppindorff tubes and stored in a refrigerator until serological test (ELISA) for bovine tuberculosis was performed. Whole blood sample (1ml) of each animal was preserved for DNA extraction and further molecular detection of organisms. Tests were performed as below:

Enzyme-linked Immunsorbent Assay (ELISA)

The optimal antigen (mammalian PPD) concentration, anti- body and conjugate dilutions were chosen after preliminary checker board titration. In the present study, the optimum conditions were 5 µg/100 µl coating buffer antigen concentration, 1:200 human and animal serum dilutions and 1:1000 Horse radish peroxidase-labeled anti-human-IgG and anti-bovine-IgG (Sigma Co.) as conjugate. The substrate used was OPD. All reaction mixture was set in duplicate, with the mean value being used for recording and calculations. Results were read on SOFT max PRO ELISA reader (Molecular Device Corporation, California) at a wave length of 405°A.

Detection of Bovine tuberculosis through molecular method

PCR for *Mycobacterium bovis*

The extracted DNA from all 100 blood samples were also subjected to PCR by using single set of primers containing forward JB21 (TCGTCCGCT GAT GCAAGTGC) and reverse JB22(CGTCCGCTGACCTCAAGAAAG) amplifying a 500 bp genomic fragment specific for *M. bovis.* The reaction was performed in a final volume of 50 µl containing 1x reaction buffer (Fermentas, USA), 2.5 U of *Taq* polymerase (Fermentas, USA), 0.2 mM each deoxynucleoside triphosphate, 1.5 mM magnesium chloride, 2 µg of each DNA and 100 pmol of each primer. Target DNA was denatured by initial incubation for 4 min at

94°C before amplification for 30 cycles of 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes. All reactions were carried out in an automated thermal cycler (Eppendorf, USA). After amplification, the PCR mixture was subjected to 1.5% agarose gel electrophoresis.

Collection and analysis of tissue samples

Tissue samples were collected from lymph node of respiratory tract, lung and liver tissue, lymph nodes from gastrointestinal from TB tested animals through tuberculin test for Lateral flow technique and Acid Fast Test. Acid fast stain (Ziehl Neelsen) test was performed by using Ziel Neeelson Staining kit containing fixatives and stain solutions. Antibodies against Bovine Tuberculosis (BT) were detected by Lateral flow technique using Rapid Bovine TB Ab test kit.

Collection and analysis of pus and caseous material

Pus and caseous material from open tuberculous cavities were asceptically collected in a sterile collecting bottle and used for culture and microscopic examination for presence of TB organism.

Collection and examination of sputum

Sputum samples for smear microscopy and culture were collected from 190 butchers, workers, and meat handlers those who voluntarily participated in this study. Prior to sample collection they were instructed to properly rinse their mouth with water as to eliminate food particles and microorganisms present in their mouth. They took deep breath and hold for 5 seconds then slowly breathe out. After another deep breathe they coughed hard until some phlegm comes into their mouth. The phlegm was collected into a 50ml sterile screw-capped bottle provided to them.Three sputum samples (spot, early morning) were collected from each participant. The sputum specimen was kept at 4^{o}C and transported to the Laboratory of Ojha Institute of Chest Diseases, Karachi for culture, and identification of isolates. Samples were processed within seven days of collection in order to minimize loss of viability of the mycobacteria.

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Data analysis

Data collected was tabulated and analysed using SPSS Statistical Software. Significant differences were mentioned as $P< 0.5$.

RESULTS

Prevalance of *Mycobacterium tuberculosis* in blood and tissues of cattle slaughetered at abbatoir in Karachi determined by Rapid Bovine TB Ab test kit and Zeihl Nelson staining of tissues. Results of the presence of *Mycobacterium tuberculosis* antibodies in serum and tissue samples are mentioned in Table 1. Eight hundred animals were examined for the prevalence of *Mycobaterium bovis* in their blood and tissues using different techniques. The results of lateral flow techniques (Rapid Bovine TB Ab) test showed highest prevalence at City Abattoir Cattle Colony in Landhi i.e 6.33%, followed by New Karachi slaughter. i.e 4.5% . The overall prevalence was recorded as 5.87%. Acid Fast Ziehl Nelson staining showed 1% and 7.33% tissues of each 150 necropsied animals from City Abattoir Cattle Colony in Landhi and 150 from the New Karachi slaughter house, respectively. A non-significant difference was recorded through lateral flow technique in the blood samples from two abattoirs having chi square value (x^2=0.91) whereas a significant difference was recorded through Ziehl Neelson test in tissue samples necropsed which has chi square value x^2=7.71.

Detection of Bovine tuberculosis by ELISA

Results of serum ELISA test of clinically suspected animals for prevalence of TB is shown in Figure 1. Out of one hundred tested blood serum samples 47% were found positive. The highest seroprevalnce was recorded in animals of age 7 years i.e. 27% and lowest 22% was recorded in 4 years. However, there was no significant difference ($P< 0.05$) in the results of different ages of animals.

Prevalance of TB by PCR assay

One hundred blood samples collected from clinically suspected cattles were analyzed for *M. bovis* and *M. tuberculosis* by PCR assay. Out of one hundred blood samples 55 were positive. The age wise percentage of prevalence of tuberculosis in cattle were 11%, 23%, 29% and 37% in age of 4, 5, 6 and 7 years, respectively (Figure 2). The results obtained through PCR method from blood samples indicate that older cattle were significantly more infected ($P< 0.05$) with tuberculosis than younger cattle.

Prevalence of bovine tuberculosis in serum of male and female cattle slaughtered

Prevalence percent was recorded of bovine tuberculosis in serum of male and female cattle

and buffaloes slaughtered in both abattoirs. Out of 800 serum samples 244 were from female and 556 were from males. Twenty four (24) of the 244 were positive for lateral flow technique giving a prevalence of 9.83 percent in the female while (28) of the 546 were positive for lateral flow technique giving a prevalence of 5.03 percent in male. Nine tissue samples out of 150 were positive for Ziehl Neelsen giving a prevalence of (6.00 percent) from female and only 1 (0.67 percent) from the male was positive for Ziehl Neelsen staining test from both abattoirs (Table 2). A significant difference was recorded through lateral flow technique in the blood samples from male and female gender having chi square value (x^2=5.24) where as a significant difference was also recorded through Ziehl Neelson test in tissue samples necropsed which has chi square value of x^2=6.62.

Table 1. Prevalence of bovine tuberculosis in cattle slaughtered at two abattoirs in Karachi

Study site	No of blood samples	Lateral flow technique positive	Lateral flow technique negative	Prevalence % age	X^2	*p value*	No of tissue samples necropsed	Ziehl Neelsen positive	Ziehl Neelsen negative	Prevalence % age	X^2	*p value*
City Abattoir Cattle Colony in Landhi	600	38	562	6.33	0.91	0.3397	150	27	123	1.00	7.71	0.0055
New Karachi slaughter house (kamailas)	200	9	191	4.50	-	-	150	11	139	7.33	-	-
Total	800	47	753	5.87	-	-	300	38	262	12.67	-	-

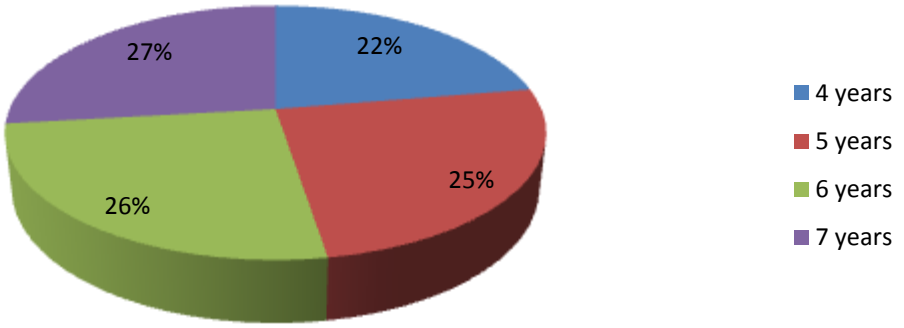

Figure 1. Age wise prevalence of mycobacterium in blood of clinically suspected cattle with ELISA

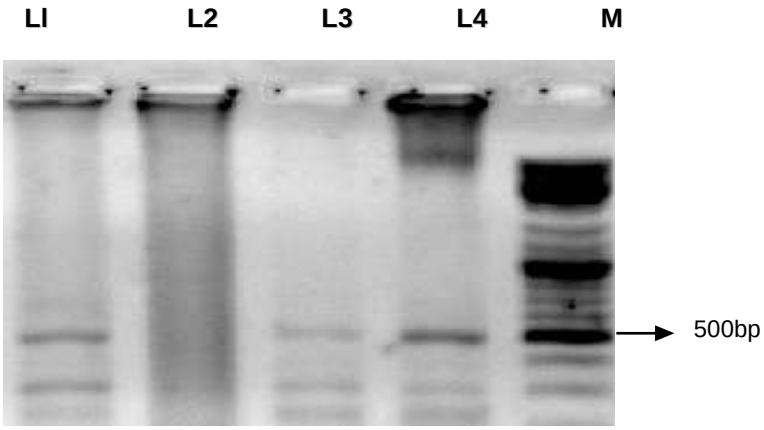

Figure 2. A 500 bp PCR product (L1, L3, L4) of *M. bovis,* obtained by using JB21 and JB22 primers in a single PCR. L2: no amplification. M, DNA marker

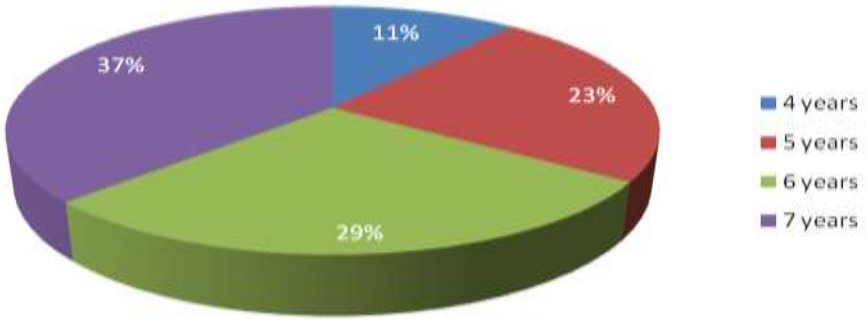

Figure 3. Age wise prevalence of mycobacterium in blood of clinically suspected cattle with PCR

Table 2. Prevalence of bovine tuberculosis in male and female cattle slaughtered at the two abattoirs in Karachi

Sex	No of samples	Lateral flow technique positive	Lateral flow technique negative	Prevalence % age	X^2	*p value*	No of tissue samples necropsed	Ziehl Neelsen positive	Ziehl Neelsen negative	Prevalence % age	X^2	*p value*
Female	244	24	220	9.83	5.2	0.0	150	9	141	6.00	6.6	0.01
Male	556	28	528	5.03	4	22	150	1	149	0.67	2	01
Total	800	52	748	6.50	-	-	300	10	290	3.33	-	-

Table 3. Microscopic results of sputum sample on Acid Fast Bacillus (AFB)

Respondent	Sputum examined	Sputum positive by AFB	Sputum negative by AFB	Prevalence (%)	X^2	*p value*
Butchers	35	5	30	14.28	6.66	0.7194
Meat handlers	20	2	18	10.00		
Workers	15	1	14	6.67		
Total	70	8	62	11.43		

Table 4. Body condition data of sero-positive cattle and buffaloes slaughtered at the two abattoirs in Karachi

Body Condition of Cattle	No. of animals examined	Rapid test positive	Rapid test negative	Prevalence (%)	X^2	p value
Poor	95	55	40	57.89	75.01	0.0000
Fair	109	41	68	37.61		
Good	141	9	132	6.38		

Table 5. Assessment of questionnaire

Variable	Number	Mean score	%age
No knowledge of the Disease	105	5.6	88.24
Work related exposure factors	9	9.7	7.56
Medical history	2	2.36	1.68
Preventive practices	3	1.9	2.52
Total	119	19.56	100.09

Table 6. Multivariate analysis of risk factor for bovine tuberculosis in butchers and meat handlers in Karachi abattoirs

Variable Exposure	Odds Ratio	Confidence Interval [CI 95%]	P - value
Length worked at the abattoir >6 / <6 years	3.7	[1.9 -7.7]	p<0.001*
Do you know the mode of transmission- Yes/ No	2.6	[1.3-5.3]	p>0.05
Do you keep animals at home- Yes/ No	1.9	[0.9-4.7]	p>0.05
Are you aware of bTB in wild animals- Yes /No	1.6	[0.9-3.3]	p>0.05
Potential risk associated With your job- Yes /No	2.7	[1.3-5.3]	0.02*
Do you eat or drink at workplace - Yes /No	0.9	[0.5-1.7]	p>0.05
Seen a BTB lesion in slaughtered animal- Yes/ No	0.4	[0.3-0.7]	0.0001*
What's done to lesion-call meat inspectoer/ Ignore	2.4	[1.2-5.0]	*p<0.05
Do you consume meat with these lesions- Yes/ No	2.5	[1.4-5.0]	* p<0.05
Have/had persistent cough- Yes/ No	1.9	[0.8- 4.3]	p>0.05
Vaccinated with BCG- Yes/ No	0.7	[0.3-1.4]	p>0.05
Received any training on BTB- Yes/ No	0.8	[0.3-1.7]	p>0.05

* Values that remained significant in the unconditional logistic regression model

Prevalence of tuberculosis in sputum of workers of slaughter-house

Sputum samples collected from butchers, meat handlers and workers at abattoirs were tested for TB under Acid Fast Bacillus technique (AFB). The high number of AFB positive samples were observed in butchers (14.28%), followed by meat handlers (10%) and workers (6.7%) (Table 3). A non-significant difference was recorded in the sputum samples examined from abattoirs respondents having chi square value of x^2=0.66.

Comparision of Sero prevalence with BCS of cattle and buffaloes

Table 4 shows body condition data of sero-positive cattle and buffaloes. Fifty five out of 95 cattle with the poor body condition were positive for rapid test giving a prevalence of 57.89%, (41) out of 109 cattle with fair body condition were positive, giving a prevalence of 37.61% and (9) out of 141 cattle with good body condition were positive giving a prevalence of 6.38%. A highly significant difference was recorded through rapid test in the blood samples from animals possessed different body conditions having chi square value x^2=75.01.

Analysis of questionnaire

Response of 119 out of 150 butchers and meat handlers on questionnaire was analyzed. Result revealed that majority of the butchers had no knowledge of disease i.e 88.24, followed by 7.56th work related exposure factors (Table 5).

Multivariate analysis of risk factor for prevalence of TB in butchers and meat handlers

Multivariate analysis of risk factors revealed that those workers who stayed more than 6 years at the abattoirs were 3.7 times more susceptible (P< 0.001) to TB disease compared to those stayed less than 6 years (Table 6). Similarly, those workers who were not know houses of the mode of transmission of TB and its potential risk associated with their job were 2.6 and 2.7 times more than those who were aware. Animals raised at home were associated with low risk of disease (P> 0.05) compared to those did not raise animals at home. Similarly, lower chances of disease (P> 0.05) among the workers who were not aware of bTB in wild animals (OR=1.6) and eat or drink (OR=0.9) at work place Table 6. Those workers who observed lesion in slaughtered animals, reported lesion to Meat Inspector and consumed meat of affected animals were: 0.4, 2.4 and 2.5 times more at risk. Those abbatoir workers having or had persistent cough (OR=1.9), not immunized with BCG (OR=0.7) and not received bTB training (OR= 0.8) were at lower risk of disease.

DISCUSSION

Risk of increased or decreased prevalance of zoonotic disease mainly depends on the population of infected animals intact with healthy animals. Thicken the population more will be the chance of transmission of infection from animal to animal. Human zoonosis of animal disease depends on number and frequency of the farmers with animals. Morover, peoples inolve in slaughetering animals are more prone to infections of zoonotic importance such as bovine tuberculosis. In present study blood and tissue samples of suspected animals slaughtered at abbatoir in Karachi were analysed by different techniques and the results were correlated with their risk of transmission to people working with suspected animals. Serum analysis with lateral flow technique revealed a prevalence of 5.87% as shown in Table 1. This is lower than the prevalence value of 12.5% using the same lateral flow technique reported by Dan Birnin *et al*. (2010), the difference may be due to number of animals and location. The prevalence of 12.67% was revealed based on the visual identification of lesions in tissues of cattle slaughtered as shown in Table 1, is higher to prevalence of 2.8% reported by Igbokwe *et al*. (2001). The difference may be due to the number of animals used for the study and for recognition of bovine tuberculous lesions in particular depend on the workload and time. Despite this, recognition of tuberculous lesions in slaughter houses can be affected due to early infection or parasites, non-specific reactions and due to the infection other than *M. bovis* as reported by Corner (1994) and other irregularities of abattoir meat inspections as was documented by Edwards *et al*., (1997).The tissue stained with Ziehl Neelsen revealed a prevalence of 12.67% as 38/300 tissue samples were positive as shown in Table 1. This is lower than a prevalence value of 31% reported by Awah Ndukum *et al*. (2010). The difference could be due to the proportion of the tissues with lesions detected. The prevalence from this study suggests that meat being sold for public consumption is either diseased or exposed to several levels of contamination which could not danger the public health. The risk of disease transmission from abattoir to the public at large is very high.

The seroprevalence was more in female 9.83% than in males 5.13%, prevalence due to visual identification lesion on tissues 3.69% in females and 0.18% in males as well as in the Ziehl Neelsen as shown in Table 2. This is consistent with the herd prevalence of 50% reported by Firdessa *et al*. (2012) where 94% of the positives were females. This demonstrates husbandry conditions as a major impact on the prevalence of bTB as reported by Ameni *et al*. (2006). Intensification, stress and over crowding are possible clarifications for such association. The same report of higher prevalence in female than male was revealed in a study by Maxwell *et al.* (2012). Cows with minimal lung lesions may spread the disease in the entire herd. Adults' cattle are usually infected through the feces, inhaling invisible droplets containing bacteria in to their lungs, and close contact while calves are infected by drinking milk from affected mother.

The seroprevalence of bTB according to the body score of the cattle was 57.89%, 37.61% and 6.38% for Poor, Fair, and Good body conditions, respectively. This is similar to a study reported by Firdessa *et al. (*2012) where the body condition scoring of the approximately 3,000 animals proposed no significant differences between tuberculin reactors and non-reactors and a poor association was also perceived among the 36 slaughtered animals.

This is consistent with findings from the few studies that evaluated knowledge and behavior among the risk groups in parallel settings in other African countries (Swai *et al*., 2010) who measured the knowledge and practice of animal health employees and livestock owners in Tanzania. They determined that, insufficient awareness (53.2%) and lack of knowledge of zoonoses (46.8%) combined with food consumption habits and poor animal husbandry are likely to expose respondents to an increased risk of contracting zoonoses. Amenu *et al*. (2010) also documented the lack of accurate knowledge on transmission of zoonoses and the prevalence of risky behaviour, as consumption of raw animal products and unsafe defeating performs in a rural district in Ethiopia.

Similarly, Mfinanga *et al.* (2003) investigated the level of knowledge and prevailing practices in rural Tanzania and showed that about 40% of respondents practice habits deemed to be high risk for exposure to bTB, while 75% showed deprived knowledge of TB.

Diagnosis of TB is a great challenge in developing countries. Signs and symptoms like fever, cough, weight loss, abdominal pain and loose motion are important features for diagnosis of TB (Chesnutt and Prendergast, 2007). History of TB contact, overcrowding in houses, younger age group, and comorbid diseases, animal handling along with poor economical conditions are important factors in spread of the disease. Sputum for AFB, X-ray chest, Monteux test, changes in blood CPC, ESR and histopathology of specimens are considered to be important diagnostic tests (OIE, 2004). However, the staining of the Sputum smear is believed to be the most significant diagnostic tool for TB but it has very low sensitivity. In Pakistan only 34% chest TB cases are sputum smear positive (WHO, 2003). To visualize the TB bacilli with a 100x microscope objective, there is need of >10,000 organism per ml of sputum.There are always chances of getting false negative resuts due to presence of dead organisms that do not take staining. (Harries *et al*., 1996).

CONCLUSION

Results of the present study concluded that there is a potential risk of zoonotic transmission of tuberculosis of animal origin to the human population working with live animals or at slaughter houses. Famale and old age animals are more prone to bovine TB. The risk factor is directly proportion to the time and duration of exposure. Molecular dection method is more sensitive and specific for the diagnosis of bovine tuberculosis.

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