Prevalence of Tuberculosis in Buffalo and Cattle

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To study the incidence of *bovine tuberculosis* in buffaloe and cattle in abattoirs of Peshawar, Khyber Pakhtunkhwa, The suspected lung and liver samples were collected at random from buffaloe (Bubalus bubalis) and cattle (Bos taurus) from abattoirs of the city and were analyzed in the laboratory of Veterinary Research Institute (VRI) for the presence of *Mycobacterium bovis* through acid fast staining, culture and polymerase chain reaction (PCR). A total of 151 animals including 121 buffaloe and 30 cattle samples were analyzed in this study from July to November 2011. Seventeen (14.04%) of the 120 buffaloe were found positive for AFB, while the remaining one hundred and four buffaloe (85.9%) were negative. Amongst the cattle only four were positive out of 30 (13.33%) for *bovine tuberculosis*. Out of the total 151 animals, 302 samples were collected, one each from lung and liver respectively and 27 (8.94%) were found with AFB. It was observed that 13.90% of the lung tissue were AFB positive while 6 (3.97%) out of 151 liver samples showed the AFB incidence. The AFB culture technique was effective in detecting the presence of *Mycobacteria*, as 8.94% of the overall suspected tissue samples were found AFB positive. The detailed laboratory examinations such as, PCR and other biochemical tests to supplement the cultural identification are recommended for accurate and reliable identification of *bovine tuberculosis* among the cattle. The microscopic examination can only determine the acid fast organisms in the suspected samples whereas culture on stone brink media along with biochemical and PCR techniques can differentiate between acid fast *Mycobacterial* species.

Key words: Buffaloe, cattle, bovine tuberculosis, incidence, lungs, liver.

Pakistan is being an agricultural country has a large livestock populations well adapted to the local environmental conditions. The national herds consist of 33.0 million heads of cattle and 29.9 million buffaloe. Livestock produce 1.601 million tons of beef[1]. *Bovine tuberculosis* remains one of the most destructive diseases of cattle in developing countries throughout the world[2]. *Mycobacterium bovis* (*M. bovis*) is the causative agent of *bovine tuberculosis* (BTB) and belongs to the *Mycobacterium tuberculosis* complex (MTBC) of bacterial strains[3, 4]. *M. bovis* is a slow growing (16 to 20 hour generation time) facultative intracellular, aerobic bacteria[5, 6] and is non motile and non capsulated acid fast bacteria having no endospores[7]. The BTB is one of the most important zoonotic diseases known to humans[8]. It is spread through air, when the infected people cough, sneeze, or spit[9]. *Bovine tuberculosis* is characterized by the formation of granulomas in tissue especially in the lungs, lymph nodes, intestines, liver and kidneys[10]. Throughout the 19th and early 20th century, a substantial proportion of human *tuberculosis* cases were caused by infection with *M. bovis* generally linked to the consumption of raw cow milk[11, 12]. *Bovine tuberculosis* diagnosis relies on radiology i.e commonly chest X-rays, a tuberculin skin test, blood tests, as well as

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microscopic examination and microbiological culture of body fluids[13]. Based on studies of slaughtered animals, the incidence of BTB in cattle and buffaloe in Pakistan has been reported to be varying from 2.25 per cent in1969 to 7.3 per cent in 1989[14]. In the Punjab (Pakistan), prevalence of BTB varies in Nili Ravi buffaloe from 5.48% to 12.72%[13]. However, no significant data is available about the situation of disease in animals of Khyber Pukhtunkhwa province of Pakistan. The low case detection rate observed in the Eastern Mediterranean region is mainly because of low detection rate in Pakistan and Afghanistan. If this remains unexplored, then a major outbreak of TB can be expected in Pakistan[15]. The present study was designed to know the incidence of the disease in buffaloe and cattle in abattoirs of the Peshawar, Khyber pukhtunkhwa.

MATERIALS AND METHODS

The present study was conducted in the Veterinary Research Institute Peshawar (VRI). For this purpose a total of 302 samples were collected from abattoirs of Peshawar from buffaloe and cattle. The gross lesions were observed in the lung and liver in postmortem. Among 302 tissue samples, 242 (121 each lung and liver) were from buffaloe and the rest of 60 (30 each lung and liver) samples were taken from cattle by the use of steriled forecep and scissor and were collected in steriled glass bottles, labeled accordingly and transported to the laboratory in an ice box within recommended time period and then processed for further studies

Processing of Samples

The collected sample was homogenized using an electrical homogenizer. The homogenized samples were transferred to centrifuge test tube. About 10 ml of 4% NaOH was added to each test tube, shaked thoroughly and left for 10 minutes. Then the samples were centrifuged at 3000 rpm for 10 minutes. The supernatant were discarded and 10 ml of fresh normal saline solution was added to each test tubes. After proper mixing the test tubes were again centrifuged at 3000 rpm for 10 minutes. This process was repeated 3 times to completely neutralize the sample. The supernatant were discarded and the sediment was used for ZN staining and culture. Furthermore, cultural growth was subjected to polymerase chain reaction (PCR) for the confirmation of *Mycobacterium bovis.*

The ZN staining technique was employed as a primary diagnostic tool to detect acid fast bacilli and the Smear was prepared from calcified tissue samples for observation of AFB.

All the samples were cultured using stone brink medium (fig 1). Slants were prepared and inoculated with the materials and incubated aerobically at 37 ^{0}C for 8 weeks.

The purified genomic DNA isolated from culture was subjected to polymerase chain reaction (PCR) for the confirmation of *Mycobacterium bovis* by using specific primer i.e farward and reverse JB21 (5'-TCGTCCGCTGATGCAAGTGC-3') and JB22 (5'-CGTCCGCTGACCTCAAGAAG-3');

RESULTS AND DISCUSSION

In the current study, lung and liver samples of buffaloe and cattle collected from different abattoirs of Peshawar city and were screened for *Mycobacterium bovis* (*M. bovis*)*,* to assess the hygienic quality and standards of meat being consumed by the public in this particular

Fig. 1. culture growth of *M.bovis*
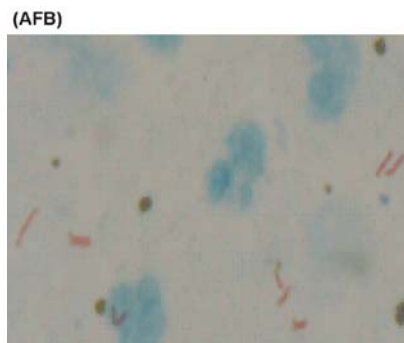
Fig. 2. Determination of *bovine tuberculosis* through culture on stone brink medium

area. Both in lung and liver samples the indicator was *M. bovis* as their presence in animal's tissues represents a health concern because of the infected animals. A total of 302 tissue samples, both lung and liver were randomly collected from 121 buffaloe and 30 cattle during the period of 5 month from June 2011 to November 2011. The samples were analyzed for the presence of *M. bovis* by

Table 1. Incidence of bovine tuberculosis in buffaloe and cattle through ZN staining in abattoirs of Peshawar

Abattoirs	Animal Species	Lungs			Livers			Overall incidence		
		Total	Positive	%	Total	Positive	%	Total	Positive	%
1	Buffaloe	54	11	20.37	54	2	3.70	108	13	12.03
	Cattle	13	2	15.38	13	1	7.69	26	3	11.53
	Sub-total	67	13	19.40	67	3	4.47	134	16	11.94
2	Buffaloe	38	6	15.78	38	2	5.26	76	8	10.52
	Cattle	11	1	9.09	11	0	0	22	1	4.54
	Sub-total	49	7	14.28	49	2	4.08	98	9	9.18
3	Buffaloe	29	3	10.34	29	1	3.44	58	4	6.89
	Cattle	6	1	16.66	6	0	0	12	1	8.33
	Sub-total	35	4	11.42	35	1	2.85	70	5	7.14
Gross Total		151	24	15.89	151	6	3.97	302	30	9.93

Table 2. Incidence of bovine tuberculosis in buffaloe and cattle through culture in abattoirs of Peshawar

Abattoirs	Animal Species	Lungs			Livers			Overall incidence		
		Total	Positive	%	Total	Positive	%	Total	Positive	%
1	Buffaloe	54	9	16.66	54	2	3.70	108	11	10.18
	Cattle	13	2	15.38	13	1	7.69	26	3	11.53
	Sub-total	67	11	16.41	67	3	4.47	134	14	10.44
2	Buffaloe	38	5	13.15	38	2	5.26	76	7	9.21
	Cattle	11	1	9.09	11	0	0	22	1	4.54
	Sub-total	49	6	12.24	49	2	4.08	98	8	8.16
3	Buffaloe	29	3	10.34	29	1	3.44	58	4	6.89
	Cattle	6	1	16.66	6	0	0	12	1	8.33
	Sub-total	35	4	11.42	35	1	2.85	70	5	7.14
Gross Total	151	21	13.90	151	6	3.97	302	27	8.94	

Table 3. Concordance pattern and overall BTB incidence with PCR results.

Abattoirs	Animal Species	Total	Positive	%
1	Buffaloe	108	11	10.18
	Cattle	26	3	11.53
	Sub-total	134	14	10.44
2	Buffaloe	76	7	9.21
	Cattle	22	1	4.54
	Sub-total	98	8	8.16
3	Buffaloe	58	4	6.89
	Cattle	12	1	8.33
	Sub-total	70	5	7.14
Gross Total		302	27	8.94

conventional methods including Ziehl-Neelsen (ZN) staining, culturing and polymerase chain reaction (PCR).

Ziehl-Neelsen staining for observing the incidence of *bovine tuberculosis* in buffaloe and cattle

The gross total of all positive buffaloe in all the 3 abattoirs were 16.52% (20/121) and all positive cattle for BTB were 13.33% (4/30) (fig 2). The overall positive animals were therefore found to be 15.89% (Table 1) with the incidence pattern in samples, lung and liver for buffaloe and cattle (Table 1) (Fig 3).[16] inspected abattoirs from major cities which revealed that *tuberculosis* detection

rate in cattle varies from 0.18% to 4.25% and the *tuberculosis* lesions were most common. Analysis of the tissues and sera samples confirmed *tuberculosis* in 31% through Ziehl-Neelsen staining, 51 % by culture and 60% on antibody detection in the cattle. Adu-bubi *et al*[17] collected 64 lungs tissue and 95 lymph nodes of which 70.3% of the lungs tissue were acid fast bacilli positive and 69(72.6%) out of the 95 lymph nodes were also observed positive for *tuberculosis*. The ZN microscopy was effective in detecting the *Mycobacteria* as 73.1% of the suspected samples were having acid fast bacilli, culture were further processed for confirmation of BTB.

The incidence of *bovine tuberculosis* (BTB) in 54 buffaloe in abattoir 1 was 20.37% i.e (11/54) and in 13 cattle was 15.38% (2/13). The overall positive animals in abattoir 1 were 16.41% (11/67), while in abattoir 2 the incidence of BTB in

buffaloe was 13.15% (5/38) and for cattle 9.09% i.e (1/11) were positive. Therefore the total positive animals in abattoir 2 were 12.24% (6/49). While the incidences of BTB in buffaloe in abattoir 3 were found as 10.34% (3/29) and in cattle, it was 16.66% (1/6), thus the overall positive animals in abattoir 3 were 11.42% (4/35). Gross total of all positive buffaloe in all the 3 abattoirs were 14.04% (17/121), and all positive cattle were 13.33% (4/30), so the gross total of overall positive animals were 13.90% as specimen (21/151) (Table 2) (fig 4). The [18] examined 3381 slaughtered cattle for *tuberculosis* lesions. Out of 150 animals 114 lung (76%) and 27 lymph node samples (18%) were positive, 96 (64%) of the tissue were acid fast positive.

Detection of *bovine tuberculosis* in tissue samples of buffaloe and cattle by polymerase chain reaction (PCR)

All the samples were subjected to PCR analysis for confirmation of BTB in buffaloe and cattle .Out of 151 buffaloe 17 (14.04%) and 4 in 30 cattle (13.33%) were positive for BTB. So a total of 151 animals 21 (13.90%) including buffaloe and cattle were positive for BTB. The PCR amplification using specific primers JB21/JB22 afforded sensitive and specific detection of a 500 bp PCR product from *M. bovis (fig 3)*. The specific 500 bp PCR products were visualized on ethidium bromide stained agarose gel (Table 3) (fig 5). De Araujo *et al.*,[19] in one of his studies observed that seventy two carcass samples as selected during slaughter inspection procedures in abattoirs of the state of Mato Grosso do sul, Brazi that seventeen (23.6%) of samples showed colonies suggestive of
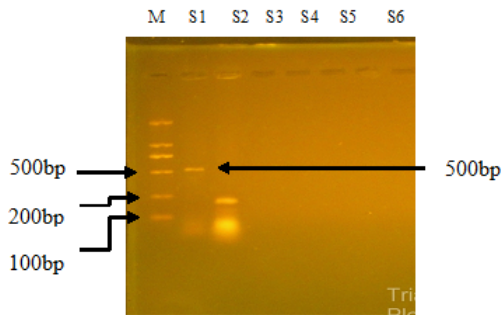
Fig. 3. Detection of *bovine tuberculosis* in tissue samples of buffaloe and cattle by polymerase chain reaction (PCR)
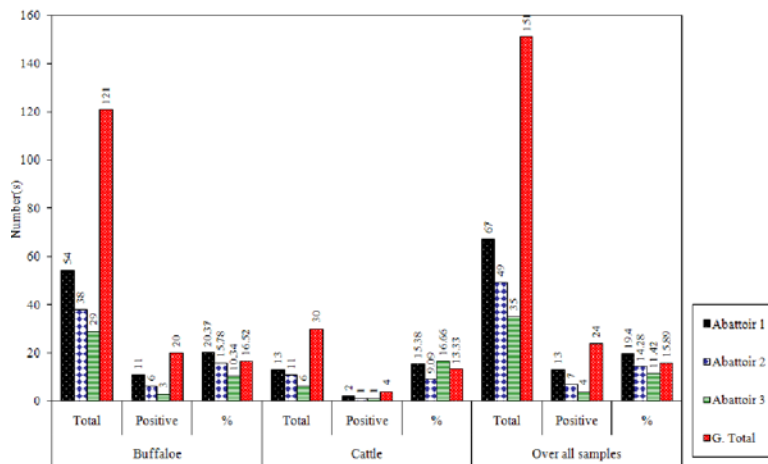
Fig. 3. Incidence of bovine tuberculosis in buffaloe and cattle through ZN staining in abattoirs of Peshawar
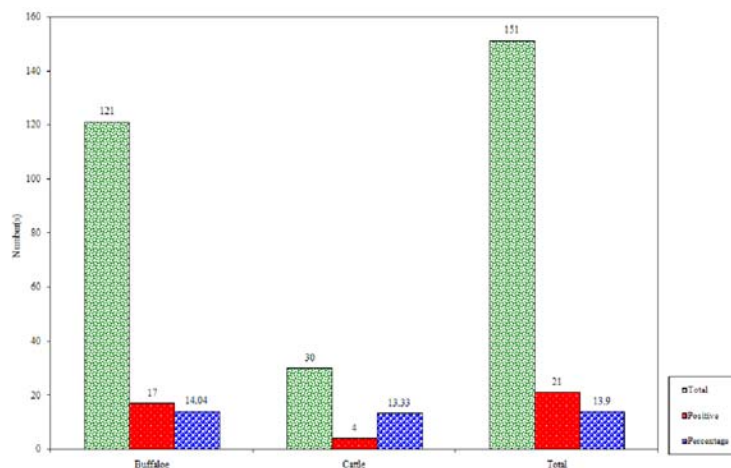
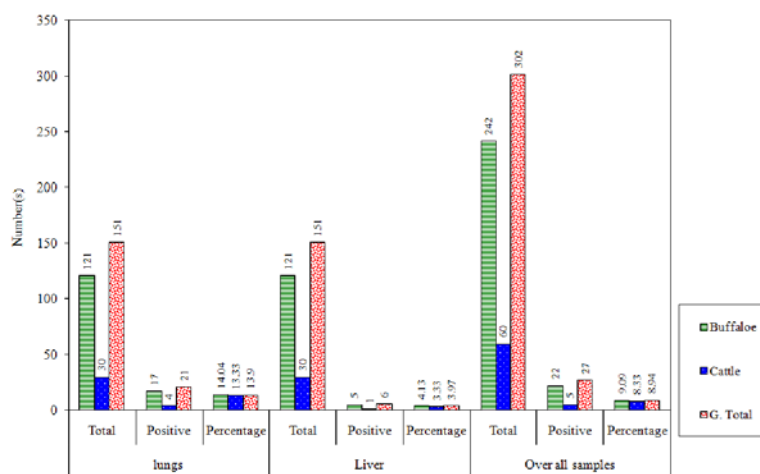**Fig. 4.** Incidence of bovine tuberculosis through culture in buffaaloe and cattele

Fig. 5. Incidence of bovine tuberculosis through culture in organs of buffaaloe and cattele

Mycobacteria growth that were confirmed to be the acid fast bacilli by Ziehl-Neelsen staining. In this case also the polymerase chain reaction (PCR) using primers specific for *M. bovis* identified the organism in 13(76.5%) isolates.

CONCLUSION

Our result suggest that the impact of BTB on animal's health, higher for pulmonary TB. The incidence of bovine tuberculosis is significant among buffaloe and cattle due to the lack of preventive treatment measures and the diseased animals in abattoirs indicate lack of quality veterinary inspection and monitoring for the prevention and control purposes.

Ante-mortem examination of buffaloe and cattle could be made more reliable on regular basis since they are mainly conducted when an animal shows signs of illness for *tuberculosis*. This will reduce the losses not only to livestock but also address the public health concerns for the needs of standard quality of meat due to its hygienic importance.

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