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RESEARCH ARTICLE

Molecular Based Epidemiology of Bovine Pulmonary Tuberculosis – a Mortal Foe

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ABSTRACT

Some epidemiological aspects and pathological alterations particularly an uncommon liver (peliosis hepatis) in association with chronic pulmonary tuberculosis in cross bred cows were evaluated. Animals were screened through single comparative cervical intradermal tuberculin (SCCIT) test. The results revealed 7.47% prevalence of bovine tuberculosis (BTB). The frequency analysis revealed that the prevalence of disease was non-significantly different with age, sex and lactating status of animals. Necropsy revealed multiple tubercles of variable size throughout the lungs. Trachea and bronchial tree was extensively plugged with thick yellow ropy purulent exudates. Microscopically lungs exhibited characteristic small and large size nodules with caseated center and lamellar arrangement of granter caseated that was surrounded by multinucleated giant cells, epithelioid macrophages, plasma cells, and lymphocytes and fibrous in Lung tissues stained with Ziehl-Neelsen expressed masses acid-fast bacilli in the necrotic centers. Moreover, in liver sections angiomatoid cysts (peliosis hepatis) lined with hepatocytes were extensively observed. The infectious agent (*Mycobacterium bovis*) was confirmed using PCR. It was concluded that histopathological observations such as peliosis hepatis are useful information for the differential diagnosis of acute verses chronic process of bovine tuberculosis.

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INTRODUCTION

Tuberculosis is one of the world's most lethal infectious diseases that inflict more than 8.6 million people (Anonymous, 2013; Gul *et al*., 2013; Siddiqui *et al.,* 2013). Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* (*M. bovis*), is a complex, destructive and zoonotic disease affecting variety of domestic and wild animals. Several reports are available about tuberculosis in accessible literature in various animal species including large ruminants (Bradshaw *et al*., 2012; Blake and Donnelly, 2012), small ruminants (Boukary *et al*., 2012), zoo animals (Gortazar *et al*., 2011; Fitzgerald and Kaneene, 2013) and captive herd of wapiti (*Cervus elaphus nelsoni*) in Colorado (Rhyan *et al.*, 1992).

The disease is usually transmitted by direct contact with infected animals, ingestion of contaminated animal products and numerous other factors including housing, feeding, through the colostrum/milk to calves and watering of infected and non-infected animal together. *M. bovis* causes chronic contagious debilitating infection in animals and has public health importance (Arshad *et al.,* 2012; Hashmi *et al.,* 2013). The disease also has high significance to international trade of livestock and its products (Anonymous, 2004). BTB commonly affects the older animals and mainly confined to respiratory system. The affected animals show progressive weight loss, tubercle (granuloma) formation primarily in lungs and occasionally in other tissues. The BTB poses threat to the public health and to the economies of developing (Rahman and Samad, 2008) as well as developed countries (Khan *et al*., 2008). The disease may also involve other tissues including liver, intestines, uterus and testes, however, CNS involvement has not been reported (Lan *et al*., 2001). Animals infected with pulmonary tuberculosis usually show respiratory distress and moist cough.

The prevalence of BTB in dairy animals has been varies in intensity and frequency from time to time under different housing and management conditions. The

prevalence of BTB in Pakistan has been reported to be 0.51 to 12.72% (Khan and Khan, 2007; Khan *et al*., 2008; Javed *et al.,* 2012). In Pakistan, different studies have been carried out to determine the association of different risk factors in naturally occurring tuberculosis in buffaloes (Arshad *et al*., 2012). However, no study has reported the pathological findings in morbid tissues of naturally occurring cases of BTB in cattle. Therefore, the present study was conducted to access the prevalence of tuberculosis and pathological findings of morbid tissues collected from animals died of BTB at a local dairy farm.

MATERIALS AND METHODS

The present study was conducted on 107 crossbred (Holstein X Sahiwal) cattle kept at the Livestock and Dairy Farm, University of Agriculture, Faisalabad, Pakistan. The prevalence of BTB was measured by SCCIT test (Ameni *et al*., 2000). Data of all the animals including sex, lactation status and age were collected. The milk samples from positive animals were collected.

Animals died (n=5) with history of respiratory symptoms were subjected to postmortem examination adopting the precautionary measures. Grossly lesions were recorded and tissue specimens from lungs and liver were collected and preserved in 10% neutral buffered formalin. Fixed tissues were processed using paraffin method, 5 µm sections were cut and stained with hematoxylin and eosin (Mashkoor *et al*., 2013). Lesions suggestive of tuberculosis were additionally stained by Ziehl-Neelsen method for acid-fast bacteria (Sikandar *et al*., 2012).

PCR Assay: The collected samples of milk were subjected to PCR for the confirmation of *M. bovis* as causative agent. DNA was extracted using Nucleic acid extraction kit (Vivantis, USA) following the manufacturer instructions. PCR was carried out to amplify the most virulent MPB70 gene of *M. tuberculosis* using specific primers (TBIF 5' GAACAATCC GGAGTTGACAA 3' and TBIR 5' AGCAGCCTGTCAATCATGTA 3'). The thermal cycler conditions optimized for the reaction (Estrada-Chavez *et al*., 2004). The amplified PCR product was electrophoresed using 100 bp DNA ladder as molecular marker and visualized in gel documentation system.

Statistical analysis: The data thus collected were analyzed by using SAS (2004) statistical software version 9.1 which included Mantel Haenszel chi-square and frequency analysis. $P \leq 0.05$ was considered as significance level. Odd ratio and 95% confidence interval was also determined.

RESULTS AND DISCUSSION

On the basis of SCCIT test overall prevalence of BTB was 7.47% (Table 1). In relation to sex, age and lactating status, a non-significant difference in prevalence of BTB was recorded. However, higher prevalence was noted in male and older cattle. Previous studies in Pakistan reported 2.47% (Arshad *et al.,* 2012) and 10.6% (Khan *et al*., 2008) of BTB in buffaloes and 2 to 12.3% in cattle (Khan and Khan, 2007). The results of present study and the reports of previous workers indicated that the prevalence of BTB varies from year to year and locality to locality in the country.

In the present study, BTB prevalence was more in older animals as compared to younger and adult animals (P>0.42), however, Javed *et al.* (2012) reported that BTB significantly increases with age. It could be due to the reason that when the animals remain longer period at the farms the chances to get the infections also increases. It also suggests that in older animals due to increase production and in lactation number the activity of immune system is decreased which favor the infections. Similar results have also been reported (Cagiola *et al.,* 2004).

Table 1: Prevalence of bovine tuberculosis in cross-bred cattle

Species/ Sex/age	No. of Animals	Positive		95% CI	Odd Ratio/ P value
		n	%		
Sex					
Male	23	2	8.69	1.48-25.87	OR=1.24
Female	84	6	7.14	2.95-14.26	[reciprocal =0.81]
Lactation status					
Heifers	22	1	4.54	0.23-20.44	Mantel-Haenszel
Lactating	13	1	7.69	0.38-32.48	chi-sq P>0.87
Dry	32	2	6.25	1.06-19.15	
Pregnant	17	2	11.76	2.02-33.73	
Age (Year)					
2 & <2	24	1	4.16	0.2-18.88	Mantel-Haenszel
3.1-5	17	1	5.88	0.29-25.75	chi-sq P>0.42
5.1-8	23	2	8.69	1.48-25.87	
8.1-10	23	2	8.69	1.48-25.87	
10-12	20	2	10.00	1.71-29.29	
Total	107	8	7.47		

During necropsy, the lungs exhibited extensive yellow color tubercular lesions and bronchial tree was plugged with thick yellow cords of purulent exudates (Fig. 1). Liver was dark in color, enlarged and has yellow color nodules at right lobe which was hard in consistency. The other organs were normal in shape, color and consistency.

Microscopic examination of lungs revealed macro and micro tubercles with both caseating and non-caseating centers. Extensive caseous material arranged in concentric lamellar fashion was observed in mature tubercles (Fig. 2 and 3). These tubercles were further enclosed within the fibroblastic rim punctuated by lymphocytes, plasma cells, macrophages and massive population of multinucleated giant cells (Fig. 4 and 5). Eosinophilic homogeneous cellular fluid was filled in the alveoli. Abundant rod shaped bacilli were visualized. As far as our knowledge is concerned, extensive bilateral granulomatous inflammation of lungs, ropy thick mucopurulent exudation in bronchial tree has never been reported previously. However, Shitaye *et al*. (2006) reported histopathological and tubercular lesions in Ethiopian cattle suffering from BTB. Inconsistent findings have been reported in experimentally induced tuberculosis in cattle and deer (Wangoo *et al*., 2005; Shitaye *et al*., 2006; Rodgers *et al*., 2007; Johnson *et al*., 2008) and Wapiti and red deer (Palmer *et al*., 2002; Wangoo *et al.*, 2005).

In the present study, histopathologically atypical lesions comprising of variable sizes of blood filled cysts (peliosis hepatis) were frequently observed in liver parenchyma (Fig. 6). The walls of these cysts were lined by single layer of hepatocytes. Multinucleated giant cells

Fig. 1: Lungs of the cow suffering from tuberculosis showing multiple macro and micro tubercles.

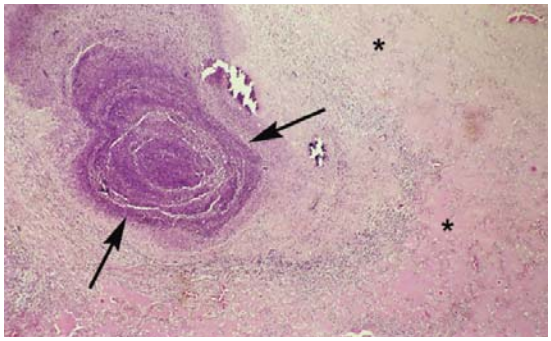

Fig. 2: Lungs of cow died of BTB exhibiting lamellated arrangement of caseous material (arrows) in mature tubercle and edema in alveoli (asterisk). H & E Stain, 100X.

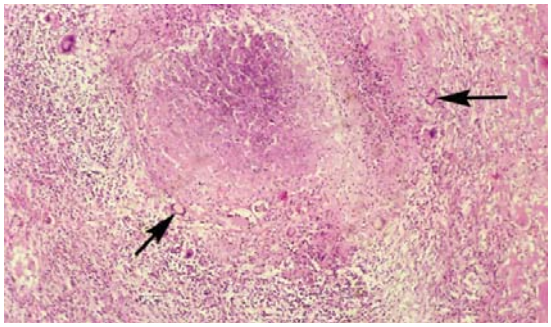

Fig. 3: Immature tubercle with large numbers of multinucleated giant cells (arrows). H & E Stain, 200X.

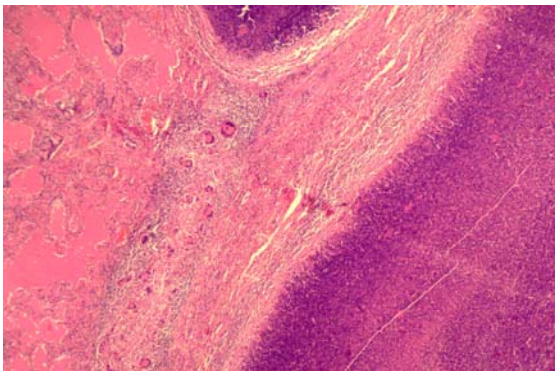

Fig. 4: Mature tubercle of lungs exhibiting granular caseous center with punctuation of brim with fibroblasts and massive population of giant cells. H & E Stain, 100X.

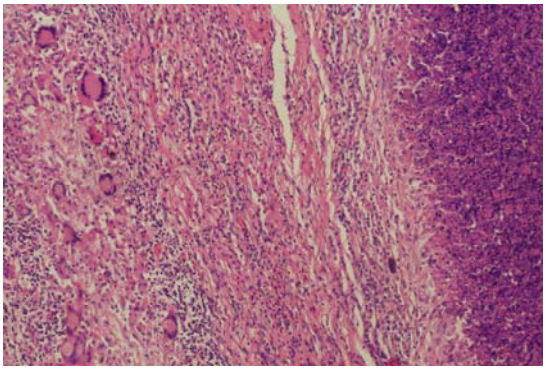

Fig. 5: Granular caseation and punctuation of fibroblasts & massive population of giant cells in lungs of BTB died cow. H & E Stain, 200X.

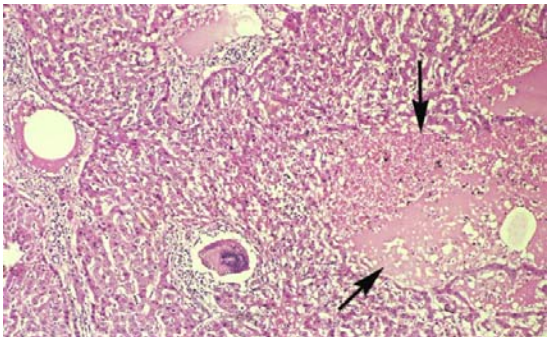

Fig. 6: Liver exhibiting multiple blood filled cyst (peliosis hepatis) of variable size (arrows) and giant cell. H & E Stain, 200X.

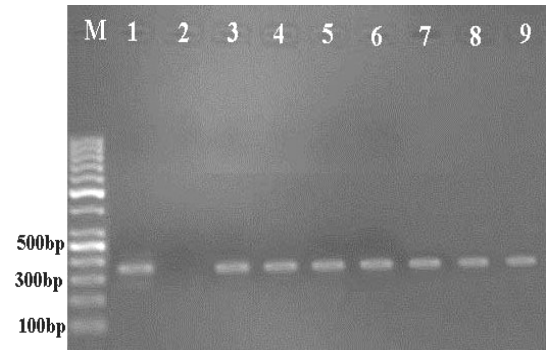

Fig. 7: Presence of 372 bp of DNA confirms *Mycobacterium bovis* in BTB suffering cattle. M: 100 bp molecular marker and lane 1-9 positive except lane 2 negative sample.

showing the engulfed material were also observed in liver. In accessible literature no report is available about peliosis hepatis in association with chronic pulmonary tuberculosis in cross bred cattle. The occurrence of multiple blood filled angiomatoid cysts in liver have been reported as a sequelae of chronic tuberculosis, steroid intake, long term use of oral contraceptives and viral infections (Gisbert *et al*., 1994; Tsokos and Erbersdobler, 2005; Kim *et al*., 2007; Motoki *et al*., 2013; Zhou *et al*., 2013). Similar blood containing cysts have been also reported in primary hepatic tumors in cattle (Bettini and Marcato, 1992).

M. bovis was confirmed by PCR in milk samples with a product approximately 372bp (Fig. 7). Previously the most virulent protein MPB70 present in *M. bovis* has been confirmed (Cousins *et al.*, 1992; Al-Attiyah *et al.*, 2003;

Estrada-Chavez *et al*., 2004; Chagas *et al*., 2010). The severity of gross and microscopic changes of this sleeping giant is serious threat to the public health.

Conclusion: The prevalence of bovine tuberculosis was 7.47% in cross bred cows. Multiple tubercles of variable size throughout the lungs with acid-fast bacilli were recorded which was also confirmed by PCR. Peliosis hepatis would be a useful information for the differential diagnosis of acute verses chronic process of pulmonary BTB.

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