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FACTORS AFFECTING PREVALENCE OF BOVINE TUBERCULOSIS IN NILI RAVI BUFFALOES

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ABSTRACT

A study was conducted to find out the prevalence of bovine tuberculosis (BTB) in Nili Ravi buffaloes at a livestock experimental station in the Punjab, Pakistan. On the basis of the comparative intradermal tuberculin test (CIDT), prevalence of BTB was found to be 10.06%. Epidemiological factors including old age, high milk production and parity played a significant role in the prevalence of the disease. Significantly ($P<0.028$) higher percentage of positive reactors was found in buffaloes aged 8 years and above (68.75%). A significantly ($P<0.043$) higher number of CIDT positive animals had parities 3-6 (68.75%) and those with milk production >7 litres per day (75.00%). A total of nine out of 16 (56.25%) animals were found positive by mycobacteriological studies. *Mycobacterium bovis* was isolated from four (44.44%) milk samples, *M. tuberculosis* from one faecal and two milk samples (33.33%), while atypical mycobacteria were isolated from two (22.22%) milk samples. It can be concluded from the present study that BTB is present in indigenous buffaloes; old age and high milk yield greatly influence its prevalence. *M. bovis* was the principal cause of tuberculosis in buffaloes. Furthermore, atypical mycobacteria also contribute to BTB to a considerable proportion.

Key words: Buffaloes, bovine tuberculosis, prevalence, milk yield, old age, mycobacteriology.

INTRODUCTION

Approximately 27.3 million heads of buffaloes place Pakistan at 2nd position after India with 98 millions and before China with 23 millions (Khan *et al.*, 2007) and contribute approximately 67% of the total milk produced in the country (Afzal *et al.*, 2007). However, their production is being hampered by various infectious diseases, like brucellosis (Gul and Khan, 2007) and tuberculosis. Tuberculosis is caused by a group of closely related acid fast bacilli forming the *Mycobacterium tuberculosis* complex. Among these organisms, *M. bovis* is most important in causing bovine tuberculosis (BTB) and has great tendency to infect human and other animals due to its wide host range (Khan and Khan, 2007).

The BTB is a chronic debilitating disease with cosmopolitan distribution. The Office International des Epizooties classifies BTB as a list B transmissible disease which is considered to be of socio-economic or public health importance and is of high significance to the international trade of animals and animal products (Cousins, 2001). The BTB is still a health problem in developed countries (Rastogi and Barrow, 1994) but is a problem of serious concern in underdeveloped countries like Pakistan, where milk is not pasteurized on large scale and disease reporting and management systems are inadequate.

The prevalence of BTB is very high in wild buffaloes and those on ranges (Jalil *et al.*, 2003), however, a high prevalence (92%) has also been reported in a domesticated buffalo herd (DeVos *et al.*, 2001). In the Punjab (Pakistan), prevalence of BTB varies in Nili Ravi buffaloes from 5.48% (Javed *et al.*, 2006) to 12.72% (Khan and Khan, 2007). In Pakistan, most of the work on BTB is confined to its prevalence as screened by tuberculin tests. The present study was designed to investigate not only the prevalence of the disease in relation to various factors but also the role of mycobacteriology for the isolation of various mycobacteria from milk and faecal samples of comparative intradermal tuberculin test (CIDT) positive and negative buffaloes.

MATERIALS AND METHODS

Experimental animals

The study was conducted on adult female Nili Ravi buffaloes (n=159) maintained at the Livestock Experiment Station, Khushab (Punjab), Pakistan over a 12 months period from July 2006 to June 2007. These buffaloes were divided into various age groups (1-5, 6-8 and >8 years), live body weight groups (<450, 450-500, 501-550 and >550 Kg), parity groups (<3, 3-6 and >6), pregnancy status (non pregnant and pregnant) and milk production groups (<5, 5-7 and >7 liters per day). Majority of the animals were reared at the farm since their birth, however, occasionally new animals from the local livestock farmers were purchased and included in the main herd in the past few years.

At farm, multiple age groups were reared but young stock was kept in separate pens. Animal groups were fed together through stall feeding and grazing. The grazing was almost at the same area where all age groups grazed together. The stall feeding was separate for each group. Each shed had a watering trough where water was available *ad libitum*. Housing pattern was mixed, i.e., open and semi-open. Animals were kept combined either indoor or outdoor and not chained. All sheds were directed either N–S or S–N. No disease outbreak occurred for the last 6 months. A village shared a common boundary with the farm premises.

These animals were vaccinated in routine against haemorrhagic septicemia, Foot and Mouth disease and black quarter. All the animals were subjected to CIDT as described earlier (Khan and Khan, 2007). Briefly, both tuberculins (bovine PPD and avian PPD) were injected intradermally (0.1 mL) on the left side of neck of each animal. The results were interpreted 72 hours post injection (Anonymous, 2004).

Mycobacteriological studies

Milk and faecal samples were collected from each CIDT positive (n=16) and 15 negative animals in separate sterile containers for microscopic examination (Cruickshank *et al.,* 1975). The udder and teats were washed and 70% ethanol was applied on the teats opening. Last few streams of milk were collected in sterile containers. Similarly, the faecal samples were collected directly from the rectum of each animal in a sterile container. These samples were stored at 4°C till further use.

All the milk samples were centrifuged at 3000 rpm for 15 minutes. Smears were prepared from supernatant and sediment, and stained with Ziehl Neelsen's stain. Similarly, faecal smears were prepared and stained with Ziehl Neelsen's stain. The milk and faecal sample smears were observed for the presence of acid fast bacilli under oil emersion magnification (Cruickshank *et al.,* 1975).

All the CIDT positive (n=16) and negative (n=15) animals were subjected to isolation of mycobacteria of various types on Lowenstein Jensen (LJ) media (glycerinated and Stone brink), smears were prepared from nasal secretions and stained with Ziehl Nielsen's method for acid fast bacilli. The milk and faecal samples were also inoculated on two types of media i.e., Stone brink and LJ media, which were prepared in screw capped culture tubes. These culture tubes were incubated at 37°C for 6-8 weeks. Cultures were examined weekly. Any culture showing no growth up to 8 weeks of incubation was considered negative. The isolated mycobacteria were subjected to culture and morphological examination along with biochemical characterization by niacin, nitrate reduction, catalase and urease tests (Cruickshank *et al.,* 1975).

The data pertaining to the husbandry and other related information for disease were collected from the record maintained at the station and through personal communication from the farm officials. The data thus generated were subjected to Chi-square test, using computer software Minitab 13.

RESULTS

Among total of 159 Nili Ravi buffaloes tested with CIDT, the overall prevalence was 10.06%. The study of various epidemiological factors revealed that greater the age ($P<0.028$), more were the chances to get infection. The prevalence of BTB differed non-significantly between pregnant and non-pregnant animals and also between various body weight groups. The prevalence of BTB in relation to milk production was the highest ($P<0.015$) in high yielding buffaloes i.e., >7 liters, followed by 5-7 and <5 liters per day (Table 1). The CIDT positive buffaloes were in higher percentage with body weight greater than 550 kg, the difference was, however, non significant.

A total of nine out of 16 (56.25%) CIDT positive animals were found positive for the bacteriological culturing. These isolates were further characterized biochemically and on the basis of cultural and morphological examination into *M. bovis*, *M. tuberculosis* and atypical mycobacteria.

M. bovis was isolated from four (44.44%) milk samples, *M. tuberculosis* from one faecal and two milk samples (33.33%), while atypical mycobacteria were isolated from two (22.22%) milk samples. *M. tuberculosis* recovered from samples showed rough, wrinkled and non pigmented colonies on LJ media containing glycerol, while none of the isolate showed growth on stone brink media. The colonies of *M. bovis* were raised and creamy white on stone brink slants.

DISCUSSION

In Pakistan, bovine tuberculosis (BTB) is prevalent in endemic proportions in livestock, humans and wildlife (Jalil *et al.,* 2003). Earlier, the prevalence of BTB in buffaloes reported from different countries varied from 0.3 to 20.2% (Khilji, 1974; Hein and Tomasovic, 1981; Woodford, 1982; Akhter *et al.,* 1992; Rodwell *et al.,* 2000; Khan and Khan, 2007). Kalema-Zikusoka *et al.* (2005) reported BTB to be 21.6% in buffaloes at Queen Elizabeth National Park, Uganda. It varied from region to region and even from farm to farm in the same region. In the present study, prevalence of BTB on the basis of CIDT was quite high. The prevalence of BTB in buffaloes is increasing day by day in Pakistan (Fig. 1) which shows the need for regular monitoring and launch programmes to control this dreadful zoonotic disease.

Table 1: Distribution of CIDT positive buffaloes in relation to various grouping

Parameters/Grouping	Prevalence		χ^2 value	P value
	No.	%		
Age (years)				
1-5	1	6.25	7.148	0.028
6-8	4	25.00		
>8	11	68.75		
Body weight (kg)				
<450	5	31.25	2.017	0.569
450-500	3	18.75		
501-550	2	12.50		
>550	6	37.50		
Parity				
<3	2	12.50	6.279	0.043
3-6	11	68.75		
>6	3	18.75		
Pregnancy status				
Pregnant	10	62.50	0.671	0.413
Non-Pregnant	6	37.50		
Milk production (liters)				
<5	2	12.50	8.466	0.015
5-7	2	12.50		
>7	12	75.00		

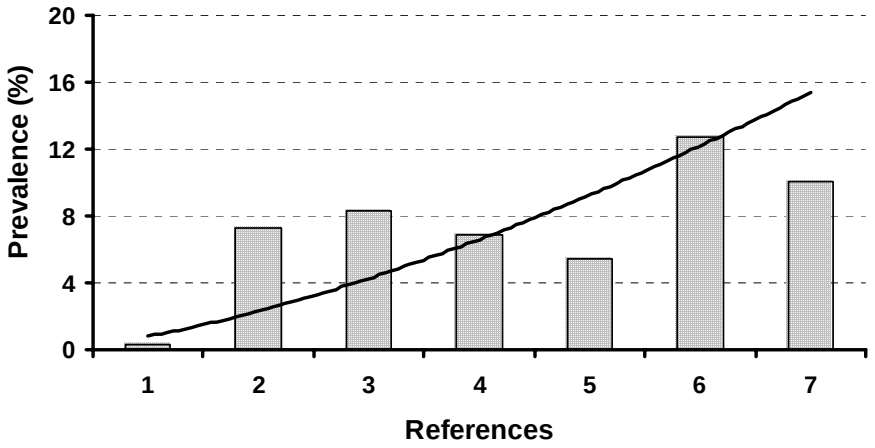

Fig. 1: Prevalence of BTB in buffaloes is increasing day by day in Pakistan (References: 1 = Khilji, 1974; 2 = Shahid, 1989; 3 = Akhtar *et al.*, 1992; 4 = Jalil *et al.*, 2003; 5 = Javed *et al.*, 2006; 6 = Khan and Khan, 2007; 7 = The present study).

The study of various epidemiological factors revealed that greater the age, more is the chance to get infection. Amin *et al.* (1992) and Rodwell *et al.* (2000) also reported high prevalence of BTB in old animals. Susceptibility of getting infection of *M. bovis* increases with age (Cagiola *et al.*, 2004) and adult cattle are more affected than calves (Kazwala *et al*., 2001). Moreover, longer span in the contaminated farm premises increases the probability of contact with the infectious agent. In addition, as the animal grows, its production of milk also increases. In the present study, it was noted that high milk producers and with greater parities buffaloes suffered more from BTB. Possible reason for this could be that high yield and pregnancy are the key factors which aggravate the disease load and animals with these factors could be immuno-compromised due to long term production stress.

In the present study, the percentage of shedding of different mycobacteria in milk and faecal samples of CIDT positive animals was 56.25%. Out of these, isolates of *M. bovis*, *M. tuberculosis* and atypical mycobacteria were 44.44, 33.33 and 22.22%, respectively. However, Ali *et al.* (2005) examined 207 (102 buffaloes and 105 cattle) milk samples, but did not

find any milk sample positive for acid fast bacilli. Neill *et al.* (1992) reported that *M. bovis* may be isolated from the nasal secretions of skin-test-negative cattle. Tadayon *et al*. (2006) reported the presence of BTB in one of the buffaloes in 140 samples collected for isolation in Iran.

From the results of present study it can be concluded that BTB is present in indigenous buffaloes due to lack of quarantine measures. Old aged animals with high milk yield greatly favour the prevalence of the disease in livestock. *M. bovis* was the principal cause of BTB in buffaloes. Mycobacteriological results revealed that milk seems to be the major source of infection for infants and consumers. Furthermore, atypical mycobacteria also contribute to the prevalence of BTB to a considerable proportion.

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