Evaluation of bovine tuberculosis by the milk secretions of cattle and buffaloes from some dairies of Quetta city

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ABSTRACT

Bovine tuberculosis (BTB) is widespread but poorly controlled in Quetta city and M. bovis is posing threats to human health. The aim of the present study deals with the evaluation of Bovine tuberculosis in the milk secretions of cattle and buffaloes. Total hundred milk samples were taken. Sixty (60) were the cattle and forty (40) were the buffaloes. In 60 samples of cattle only one case was positive. Thus, the percentage of bovine tuberculosis was 1.67% in the cattle of those dairy farms and 98.33% they were free from the M.bovis while in 40 samples of buffalo no positive case came on front therefore, the percentage for positivity in buffaloes remained 0 %. Hence, the current study reveals that the overall prevalence of Bovine tuberculosis is only 0.1% in the selected area.

Key words: Bovine Tuberculosis, cattle and buffaloes, dairy milk.

INTRODUCTION

Meat and milk are significant sources of protein and other nutrients but can be infected by pathogenic agents. The likelihood exists for the spread of tuberculosis and other mycobacterial infections from animals to humans, probably by ingestion of infected raw (unpasteurized) dairy products or meat [1, 2, and 3]. Milk itself possesses small number of micro-organism when it leaves the normal udder however, it may get contaminated from manure, water, soil, milker’s hands, utensil and flies [4]. Due to high prices of packed milk as compared to raw milk, the majority of Pakistanis purchase raw milk from milkmen and milk shops [5]. Bovine Tuberculosis caused in animals is mostly present in the milk of that infected animal which is a chronic contagious respiratory disease of cattle spreading horizontally within and between species by the aerosol and ingestion .The etiology of B.Tuberculosis is the infection caused by Mycobacterium bovis, a Gram positive, acid-fast bacterium in the Mycobacterium tuberculosis complex of the family Mycobacteriaceae [6, 7]. Although respiratory route is considered the most important for transmission of tuberculous bacilli: The alimentary route of infection plays an important role in spreading this disease because milk carries microorganisms farther into urban areas if the population still drinks raw milk [8]. M.Bovis can survive for several months in the environment, particularly in cold, dark and moist conditions.
At 12-24 Centigrade (54-75 Fahrenheit), the survival time varies from 18 to 332 days, depending on the exposure to sunlight [9]. Bovine Tuberculosis is occurring in almost all developed and developing nations of world. The incidence of disease is not only higher in the developing nations but in the absence of any national control and eradication programmes is also increasing worldwide particularly in the Asian, African and Latin American countries [10]. In humans the Mycobacterium Bovis is the major cause of extra-pulmonary tuberculosis like tuberculosis of cervical and mesenteric lymph nodes, the peritoneum and the genito-urinary tract. In countries where bovine milk is not pasteurized before use, Bovine Tuberculosis has emerged as the single major cause of extra-pulmonary human tuberculosis [11]. Animal and human tuberculosis (TB), caused by pathogenic bacteria of the Mycobacterium tuberculosis complex, M. bovis, and M. tuberculosis emerging or reemerging and [12] are prevalent and affecting the human health in Africa and animal industries [13-19]. The transmission of M. Bovis to humans via milk and its products are eliminated by pasteurization of milk [20]. Tuberculosis lesions can affect any part of the body but generally affect the lungs and lymph nodes and the chest cavity. The normally smooth internal lining of the chest cavity may be marred by lesions. Production losses in cattle occur by earlyculling, ill-thrift, and decreased milk production. Carried out a research in determining the contribution of Mycobacterium bovis to active tuberculosis in the Australian population during mid eighties to mid nineties, and also analyzed the demographic data from bacteriologically proven cases [21]. The Present study was conducted in Center for Advanced Study in Vaccinology and Biotechnology (CASVAB), Quetta for the prevalence of Bovine Tuberculosis. The study was performed by the processing of milk samples of cattle (i.e.) buffaloes and cows obtained from The Military Milk Dairy Form Quetta, Cantonment and from the some dairies of Sariab Road Quetta.

MATERIALS AND METHODS

The Present study was conducted in Center for Advanced Study in Vaccinology and Biotechnology (CASVAB), Quetta for the prevalence of Bovine Tuberculosis. The study was performed by processing the milk samples of cattle (i.e.) buffaloes and cows obtained from the Military Milk Dairy Form Quetta, Cantonment and some dairies of Sariab Road Quetta.

Collection of milk samples

Total 100 milk samples of cattle i.e. buffaloes and cows, were randomly collected from the dairy farms of Quetta City, Pakistan. Some samples were collected from few dairy farms of Sariab Road, Quetta and half of them from Milk Dairy Farm Quetta Cantonment. All the possible hygienic measures were adopted during collection and transportation of milk samples. Before sampling each teat was scrubbed with a pledged of cotton moistened with 70% ethyl alcohol. A separate pledge was used for each teat. After discarding the first few streams, about 5-10ml of milk was collected. The collected samples were immediately transferred to laboratory refrigerator at 4˚C and not for more than 24 hours.

Processing of samples

The samples were analyzed and processed with the procedure of centrifugating each sample at 3000 RPM for 15 minutes and the supernatant was discarded. The pellet was suspended in 2ml of sterilized physiological saline solution. To the suspension was added equal volume of sterilized 4-N sodium hydroxide solutions and one drop of 0.05% Phenol red indicator and the mixture incubated for 30 minutes at 37˚C. Finally the samples were neutralized with sterilized 4-N hydrochloric acid solution and were centrifuged at 3000 RPM for 15 minutes, and sediment was used for microscopic and cultural examination.

Acid Fast Staining:

The characteristic difference between Mycobacteria and other microbes is the presence of a thick, waxy (Lipoid) wall that makes the penetration of simple stain (i.e. Gram Stain) very difficult [11].

Microscopic examination:

From pellets of each sample two smears were prepared, dried, slightly fixed over flame and stained with Acid Fast stain (Ziehl-Neelsen stain). The stained smears were examined under oil-immersion lens for Acid Fast Bacilli (AFB). Acid Fast bacteria stain bright /rose red with a blue background.
Test Protocol:
The samples were stained with acid fast staining. Owing to the fact that they can’t be stained with other ordinary stains because of high lipid content of acid-fast cell walls; in particular, mycolic acid—a group of branched chain hydroxyl lipids—appears responsible for acid fastness.

Thus, they were stained by a harsher treatment: heating with a mixture of basic fuchsin and phenol (the Ziehl-Neelsen Method). When basic fuchsin got penetrated with the aid of heat and phenol then, the acid fast cells did not easily decolorize by an acid-alcohol washing hence, the positive samples remained red [22].

RESULTS AND DISCUSSION

During this study the Acid fast bacillus (AFB) was detected microscopically and isolated from the milk sample of only one cattle out of 60 cattle, while from 40 buffaloes no AFB was visualized after microscopy. Therefore, only one out of 100 dairy animals was screened for Acid Fast Bacilli (AFB). Rest of the animals were remained exempted from the discharge of Mycobacterium bovis in their milk in the chosen area of research. Of the 60 cattle, 01 (1.67%) gave positive result for Mycobacterium bovis while other 40 buffaloes showed no positive results. The complete research work depicted 1% prevalence of Bovine tuberculosis in the targeted area.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Positive cases</th>
<th>Negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Cattle</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td>Result in percentage</td>
<td>0%</td>
<td>1.67%</td>
</tr>
</tbody>
</table>

Table-1: Result of positive and negative cases after performing Acid Fast Staining

<table>
<thead>
<tr>
<th>Species</th>
<th>Areas of sample collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>LODHI DAIRY FARM, SARYAB ROAD QUETTA MILITARY DAIRY FARM QUETTA, CANTANOMENT</td>
</tr>
<tr>
<td>Buffalo</td>
<td>40</td>
</tr>
</tbody>
</table>

Graph 1: The graphical representation of milk samples taken from different dairies of Quetta, Baluchistan, Pakistan
CONCLUSION

According to present study, incidence of bovine tuberculosis is 1.67% in cattle, while 0% in buffalo. 98.33% were free from the M.bovis while in 40 samples of buffalo no positive case came on front. Hence, the current study reveals that the overall prevalence of Bovine tuberculosis is only 01% in the selected area.

Acknowledgement

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REFERENCES