

# Seroprevalence of Paratuberculosis in Cattle and Buffaloes in various Regions of Pakistan: A Comparative Study

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**Abstract:** Paratuberculosis (PTB) is a chronic disease of cattle and other ruminants and disease. It is characterized by severe diarrhea and weight loss. The affected animals eventually die after a prolonged course. Very few studies had previously been conducted in Pakistan on the prevalence of this disease. The present was aimed at knowing the prevalence of this infection in certain important districts of Pakistan. A total of 300 milk samples were collected from apparently healthy cattle and buffaloes at livestock holdings in five districts of Pakistan i.e., Karachi (n=68), Quetta (n=51), Peshawar (n=60), Lahore (n=66) and Islamabad (n=55). The milk samples were processed for separation of lactserum and then an indirect ELISA was conducted to test for the presence of antibodies in these samples against the infection. The milk samples found to be positive were only from two districts i.e. Quetta and Karachi. The samples from rest of the districts were found to be negative. The prevalence rate in Quetta was 13.73% and 2.94% in Karachi. The combined prevalence rate of both cities was 7.56%. The statistical analysis showed that the probability of the occurrence of PTB was 5.3 times higher in the district Quetta as compared to the district Karachi ( $p \leq 0.05$ ). No association of the prevalence of the disease with any other factor i.e. animal species (cattle or buffalo), age, milk yield, and farmer education was noted. Though the present study suggests a low prevalence of the disease in the major districts of Pakistan, further studies are needed to get a clear picture of the disease in the various regions of Pakistan.

**Keywords:** Paratuberculosis, Seroprevalence, Buffalo, Cattles, Pakistan, Johne's disease.

## 1. Introduction

*Mycobacterium avium* subsp. paratuberculosis (MAP) causes Paratuberculosis, a mycobacterial infection of ruminants. It starts as a local inflammation that can spread to other parts of the body, causing chronic granulomatous enteritis, which can lead to weight loss (including diarrhea in some species) and death. Johne's disease is another name for this syndrome. It might emerge as solitary clinical cases or chronic epidemics, depending on how long it has been established in a herd. The etiology of paratuberculosis is like that of tuberculosis and other mycobacterial diseases, with MAP being a specialized intracellular pathogen that causes a devastating immunopathological response; however, subclinical infection is more common (Whittington et al., 2012). Because the disease has a 2- to 10-year incubation period and many species might host the parasite without ever presenting clinical signs, finding latent instances has been difficult. Nevertheless, the lack of chemotherapeutic medicines, as well as the risks associated with vaccination, are causing concern (McKenna et al., 2006 Barry et al., 2011). Additional disorders that can be caused by MAP include winter dysentery, malnutrition intestinal parasitism, salmonellosis, and chronic molybdenum poisoning. Moreover, the disease has been termed a spectral disease, a hidden threat, and an insidious challenge for the dairy business, making eradication especially challenging. The sheep, goats, buffaloes, deer, antelopes, wildebeests, and camels are all harmed (Beard et al., 2001, Glanemann et al., 2008, Stief et al., 2012). The disease is difficult to control since the pathogen spreads freely across and between species. The general understanding of MAP's zoonotic potential is that the causal agent of paratuberculosis and human Crohn's disease are linked. The Grampositive acid-fast bacteria and member of the *Mycobacterium avium* complex group that causes a chronic, progressive, infectious infection, is a microbial illness affecting the dairy sector. Due to lowering of milk production, cow culling, decreased, infertility disease treatment, and calving rates management expenses, MAP has a negative influence

on productivity. Subclinical infections have no symptoms and sometimes go undiagnosed, resulting in a long-term decrease in milk supply. The disease is controlled through animal culling to limit disease transmission, and clinical signs include diarrhea, decreased milk supply, edema, infertility, and weight loss. Moreover, MAP could be zoonotic in origin, with a putative causal link to Crohn's disease in humans, an inflammatory disease characterized by persistent bowel wasting. The discovery of MAP in dairy food products and the intestinal tracts of Crohn's patients, as well as the pathophysiological parallels between PTB and CD, have all contributed to this association. Human exposure to MAP can occur through raw milk; however, investigations have shown that MAP can survive pasteurization procedures, providing processed milk a reasonable alternative too. The presence of MAP in the milk of both clinically and sub-clinically infected cows has prompted the EU to develop MAP control initiatives to protect public health safety. The presence of MAP in newborn powdered milk is a major source of worry, particularly in cases of pediatric disease of Crohn. Cows excrete MAP-laden feces, which pollute surface waterways from farming overflow, which eventually move into water system which will be consumed for drinking, providing an alternative way of spread.

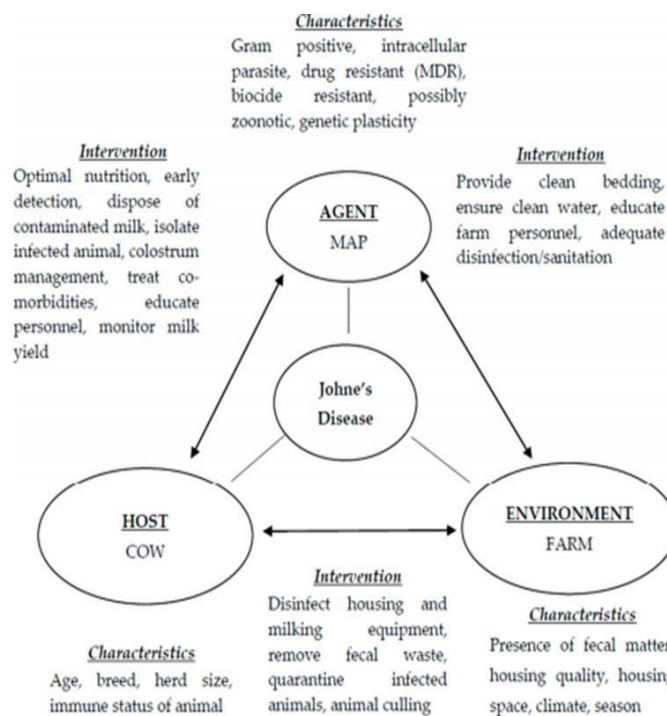


Fig 1. Graphical representation of MAP characteristics and intervention (Garvey *et al.*, 2020).

At least two different strains of MAP have been found, each with a different host preference. Type II strains (also known as type C strains) were first discovered in cattle, but they now infect sheep, goats, camelids, and ruminant and nonruminant species as well. Small ruminants are the most common carriers of type I (type S) strains, but they've also been found in cervids, South American camelids and camels, and some cattle that come into contact with sheep. Type III strains were once considered a separate "intermediate" group, but they are now considered a subtype of type I. There are two types of bison (B) strains: one for North America and the other for Asia. The latter is known as the "Indian Bison type," a sub lineage of type II. It has been discovered in cattle, water buffalo, sheep, goats, deer, bison, rabbits, wild boars, and other species, and it is the prevalent strain in Asia. In Norway, where paratuberculosis appears to predominantly affect goats and only sporadic infections in cattle and sheep have been observed, a goat-specific strain has been suggested. Other studies have been unable to prove the existence of a distinct goat strain, and management factors or breed-related resistance in cattle could plausibly explain the situation in Norway (CFSPH, Report 2003-2020).

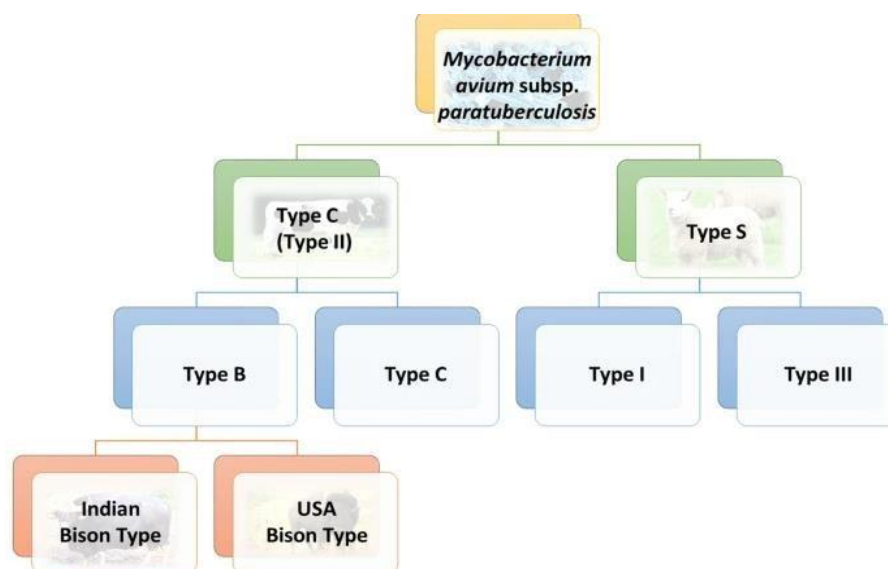


Fig.2 MAP nomenclature.

Sheep-type (Type S), Cattle-type (Type C), and a "intermediate" Type III, a sub-type of Type S, are the two primary categories of strains. Type B is a subtype of Type C that is commonly found in Bison (Matthews *et al.*, 2021).

Any pathogenic species can be transferred to humans by one of three broad methods, depending on the organism's life cycle and environmental survivability. MAP is predominantly an intestinal parasite found in a variety of species that can live for long periods of time outside of its host habitat. Until far, the majority of published research has focused on transmission routes involving watery food and zoonotic exposure (Wynne *et al.*, 2011).

### Prevalence of PTB in Pakistan

Many cases of PTB have been reported in Pakistan. In 2011, 134 probable animal samples were tested for PTB seroprevalence, with an 11.19% seroprevalence rate (Cattle: 6.67%, Buffaloes: 12.5%) (Sikandar *et al.*, 2011). PTB was found in 20% of breeding bulls and above 33% of teaser bulls evaluated from three Pakistani sperm production facilities. Many cases of PTB have been reported in Pakistan (Abbas *et al.* 2011).

## 2. Research Methodology

This study was conducted at the National Agricultural Research Center (NARC), Islamabad and the Department of Zoology, Quaid-i-Azam University, Islamabad. A total of 300 milk samples were collected from cattle and buffaloes at animal farms from the following districts of Pakistan: Quetta, Karachi, Peshawar Lahore and Islamabad. Milk was taken from animals suspected of having PTB showing signs such as (Pipe stream diarrhea, weight loss, and edema due to hypoproteinemia caused by protein-losing enteropathy and milk reduction (Sweeny: *et al* 2012). Milk samples were also collected from an apparently healthy animal.

Ten to 15ml of milk were obtained from each cow in a sterile recipient (collection vial). The samples were kept refrigerated at 4°C until they were processed. While collecting samples, data such as location, animal species (cattle or buffalo), age, milk yield, and farmer education were recorded. After sample collection should be transported to a small cooler or ice container; then, those samples were transported to the laboratory of Animal health section NARC Islamabad for processing (Harmon. *et al.*, 2010).



Fig 3. Lactoserum separated from milk

### 3. Data Analysis/Results

3.1 The results of the ELISA for detection of antibodies against MAP are shown in the table 3.1.

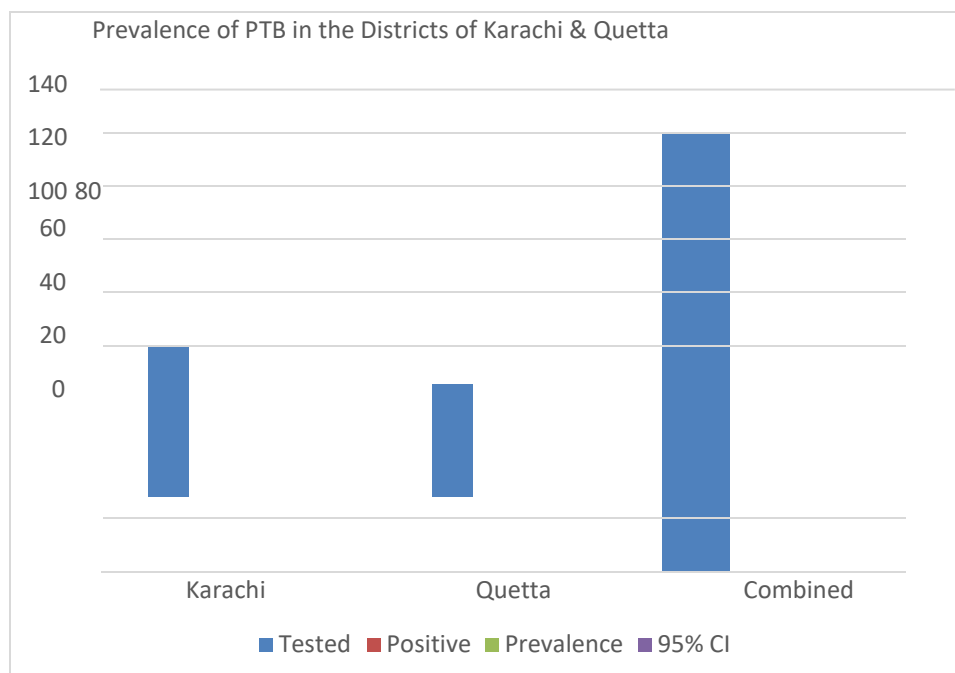
Table 1. Results of ELISA conducted over lactoserum samples collected from the five districts of Pakistan.

Serial no:	City	Total no of samples ELISA tested	ELISA Positive	ELISA Negative	%age of Positives
1	Karachi	68	2	66	2.94%
2	Quetta	51	7	44	13.73%
3	Peshawar	60	0	60	0%
4	Lahore	66	0	66	0%
5	Islamabad	55	0	55	0%
6	Total	300	9	291	3.0%

Since the positive samples were observed only in the districts of Karachi and Quetta. So, further analysis of data was performed only on the data of these two districts using the statistical analysis performed using the software Epi Info. The odds ratio revealed that the chances of occurrence of PTB were 5.3 times higher in the district Quetta as compared to the district Karachi ( $p \leq 0.05$ ). No significant association of the prevalence of the disease with any other factor i.e. animal species (cattle or buffalo), age, milk yield, and farmer education was observed.

Table2. Prevalence of PTB in the districts of Karachi and Quetta

City	Tested	Positive	Prevalence	95% CI
Karachi	68	2	2.94%	0.36-10.22
Quetta	51	7	13.73%	5.76-26.26
Combined	119	9	7.56%	3.52-13.86



#### 4. Conclusion

The present study shows the seroprevalence of PTB in the various regions of Pakistan (Karachi, Quetta, Lahore, Peshawar and Islamabad) was studied using an ELISA conducted on lactoserum extracted from milk of animals from these regions. It was found that two cities of Pakistan namely Karachi and Quetta had animals that were positive for PTB. Results showed 2.94% seroprevalence of PTB in Karachi and 13.73% in Quetta and their combined prevalence was 7.56% and no positive samples were seen in the other cities i.e. Lahore, Peshawar and Islamabad. Results showed that the chances of the occurrence of PTB was 5.3 times higher in the district Quetta as compared to the district Karachi ( $p \leq 0.05$ ). No association of the prevalence of the disease with any other factor i.e. animal species (cattle or buffalo), age, milk yield, and farmer education was predicted. There may be several reasons for the presence of infection in the milk sample. The disease might have been due to vertical transmission (i.e. in utero) or its transmission may be pseudo-vertical transmission (i.e. by milk sucking). The negative results in the three districts predict the absence of the disease in these districts. However, further studies are needed to confirm the absence or presence of the disease in the various regions of Pakistan.

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