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Comparative Seroprevalence Analysis of Paratuberculosis in Goats and Cattle: Insights and Implications

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Abstract

A chronic illness that affects cattle and other ruminants is called paratuberculosis (PTB). Severe diarrhea and weight loss are its defining characteristics. Eventually, after a protracted illness, the afflicted animals pass away. The primary site of infection for Mycobacterium avium subspecies paratuberculosis (MAP) is the intestines, where it causes intestinal wall thickening and chronic inflammation. This study's goal was to compare the seroprevalence of PTB in goats and cattle to determine the prevalence of MAP antibodies in these two different species. In six districts of Rajasthan, namely, Dausa, Bikaner, Jaisalmer, Jaipur, Udaipur and Dholpur an overall of 1951 blood specimens were taken from livestock holdings that appeared to be healthy, consisting of cattle and goats. Following the processing of the blood samples to separate the serum, an enzyme-linked immune sorbent test (ELISA) was performed to determine whether the samples had any antibodies against the infection. Only blood samples from the districts of Dausa and Bikaner were confirmed to be positive. It was discovered that the samples from the remaining districts were negative. The results of this study advance the understanding of the epidemiology of goat and cattle PTB, guiding the development of focused control measures for this disease that affects ruminant populations and has a substantial economic impact. However, this study suggests that the major areas of Rajasthan have low sickness prevalence.

Keywords: Paratuberculosis (PTB), Mycobacterium avium Subspecies Paratuberculosis (MAP), Seroprevalence, Rajasthan, Enzyme-Linked Immune Sorbent Test (ELISA), Cattle, Goat

INTRODUCTION

A localized intestinal inflammation linked to MAP. Infection is the cause of PTB, known as Johne disease there is enough data to suggest that small ruminant PTB differs from cow PTB in terms of the clinical presentation and the MAP strains involved, despite the widespread belief that the illness should manifest identically in the domestic ruminant species (1). The illness was initially discovered in cattle at Europe in 1895 and since then, it has spread to developed and developing nations, especially in the dairy as well as bovine sectors. Since the illness in small ruminants has been found in sheep and goats across several nations, it is thought to have a global spread (2). Both small and big ruminants can get PTB, a chronic wasting illness that results in significant weight loss, emaciation, chronic diarrhea, decreased milk and production of wool. Before any clinical symptoms appear, infected animals could excrete MAP in their milk and feces for up to two years. This means that vulnerable animals might come into contact with the disease via the fecal-oral route. In humans, there was a connection between Crohn's disease and MAP, however, this link is debatable (3). The World Organization for Animal Health has declared that one of the top infectious disease priorities is PTB, given the financial significance of the illness to livestock globally. There are four known phases of PTB in cattle: clinical illness, advanced clinical disease, subclinical disease and silent infection (4).

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There are no clinical symptoms of the silent illness and neither body weight (BW) increase nor bodily condition is affected. Adult carriers of the subclinical illness cannot exhibit any particular clinical symptoms, although they could have other problems, including infertility or mastitis. Despite a normal appetite, there is a progressive loss of body weight in the clinical illness and diarrhea appears a few weeks later. Vital indicators are in normal ranges, but milk production is declining (5). Globally, PTB poses a serious threat to health and the economy, particularly to the animal sector. Reduced milk production (15–16%), poor carcass yield, reduced weight growth, increased sterility, delayed parturition and early animal culling all result in financial losses. In a herd with at least 10% prevalence, the illness is projected to cause an annual economic loss of 16,674 rupees per afflicted cow. The majority of the globe is affected by PTB and its frequency is sometimes increasing (6). Numerous European, Oceanic, Asian and African nations have reported on it. Reports of the illness have come from continents in America. The frequency of MAP in cattle and other animals has been the subject of several research conducted all over the world. With the supply of vital commodities like meat, milk and other dairy products, the livestock business supports a large number of economies (7). But PTB poses a constant danger, adding a degree of complication that calls for a more sophisticated approach. By providing insights into the origin, dynamics of transmission, difficulties in diagnosing the disease and possible economic consequences, this paper seeks to clarify the existing level of knowledge surrounding PTB in goats and cattle.

The paper (8) demonstrated how a model of multivariate regression analysis using logistic was used to identify the related risk variables for MAP infections in the camels under examination. In total, 7.5% of the camels under examination had MAP infection. A logistic regression model with several variables was run to ascertain the related risk variables for MAP infection in the camels under examination. The research (9) aimed to determine the seroprevalence of MAP and risk variables by testing positive for antibodies in flocks of sheep on the dry land and Mexico's Sonora region is hot. In 43 flocks, a cross-section of research was conducted between February 2012 and December 2014. Through immune diffusion in agar-gel, serum specimens from 1178 distinct sheep were collected to identify anti-MAP antibodies. An assessment was given to the owners to gather data regarding animal and flock administration risk factors during the blood sample.The majority of research conducted in Egypt concentrated on the disease's prevalence in cattle, although a smaller number of investigations noted the presence of antibodies in the direction of MAP in sheep. The article (10) analyzed the risk variables linked to infection with MAP and found the MAP seroprevalence rate amongst sheep in four districts. Throughout 3.75% and 12.3%, the rate of seroprevalence of MAP in sheep was different throughout governorates; however, it was not significant.

The study (11) looked at the PTB prevalence in cattle in a few different Egyptian locations. The findings showed that older animals had a much greater infection rate than younger ones. Compared to other areas under study, the Gharbia governorate had a higher illness prevalence (19.6%). After 138 fecal specimens were analyzed using real-time PCR and culture, the effectiveness of the tests for diagnosis was assessed. The study (12) provided an overview of the current knowledge on the two main types of IIBD in Africa as well as animal PTB. It included a discussion of the prevalence, financial effects, epidemiology and "control of MAP along with its contribution to IIBD in Africa." While MAP was known to occur globally and has been reported in a number of African nations, much research has been done on the continent, making it difficult to evaluate its epidemiological and socioeconomic effects. The study (13) was to determine the level of PTB in cattle that were killed at the ELFORA Abattoir in central Ethiopia, using gross and microscopic lesions. Four hundred seemingly healthy cattle that were killed at the ELFORA export slaughterhouse had their tiny intestines and related lymph nodes inspected for microscopic and macroscopic PTB lesions. The study (14) first raised concerns about the general specificity of the ELISA for PTB antibodies, which led to the discovery of a fecal screening for the causative living thing, which allowed for the confirmation or denial of infection in distinct seropositive animals. Over the course of the program and the necessary re-examination of a herd, there has been a progressive improvement in the diagnostic tests and the underlying methodology for employing tests to assess the risk of PTB. Examination of PTB immunization records for cattle to see if animals had non-specific effects (NSE) on the total mortality comparable to those seen in people vaccinated with BCG. Regarding the age-related prevalence of advanced clinical manifestations of patent illness, the findings of a slaughterhouse

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investigation on PTB prevalence that was previously published served as a guide. Cows from 30 cattle ranches in Spain's Basque Country were monitored over a period of one to thirteen years as part of the appropriate vaccination research (15). The study (16) was to ascertain the genotype distribution in detail with a single herd of goats, as well as the variety of four goat herds in Thuringia, Germany, which were home to MAP that had been infected with PTB. To genotype animal faecal specimen isolated with the infection, from different digestive systems and other regions of animals that were clinically impacted and from samples of the environment, three approaches were combined. Five distinct phylogenetic groupings can be attributed to the six MAP-C genotypes that were found. The study (17) aimed to evaluate and contrast novel antigens that could address this issue. The amount of interferon-gamma (IFN-γ) produced in peripheral blood on various days following immunization in testing offspring and mature goats was evaluated in field settings using a pair of Mycobacterium bovis peptide cocktails, antigens VK 055 coupled with VK 067 of MAP, as well as Purified protein derivative (PPD) from cattle and birds. The study (18) was to evaluate the variables that can be crucial to the eradication effort and has a stronger correlation with PTB prevalence in Hungarian cattle fields. They went to 26 sizable Hungarian dairy herds that had tested positive for PTB during the previous two years and had preliminary PTB data. Ten managerial factors that might affect PTB's spread were evaluated. The study (19) examined previous research that looked for genetic markers that can be used to identify and choose calves that were more resistant to PTB and less sensitive to it. Single-nucleotide polymorphisms (SNPs), candidate genes and quantitative trait loci (QTLs) to vulnerability to MAP disease has been made possible in the past decade by genotyping and the imputation to whole-genome sequencing (WGS). There is a dearth of epidemiological information about PTB in Ethiopia, suggesting that the disease can be widespread there. Therefore, using microscopic and gross lesions from cattle killed at the ELFORA Abattoir in central Ethiopia, the purpose of the research was to ascertain the prevalence of PTB (20).

Contributions of the paper

- The research was designed to compare the seroprevalence of PTB in goats and cattle to determine the prevalence of MAP antibodies in these two species.
- The serum was separated from blood samples by processing and ELISA was used to find out if the samples contained any MAP antibodies.
- Positive prevalence outcomes were limited to the districts of Bikaner and Dausa.

METHODOLOGY

In this section, we discuss the comparative sero-prevalence analysis of PTB in goats and cattle.

Prevalence of PTB in Rajasthan

In Rajasthan, there have been several PTB instances documented. PTB seroprevalence was determined in 1951 probable animal samples, yielding a seroprevalence rate of 11.19%. 25% of breeding bulls and more than 40% of teasing bulls analyzed from three sperm production plants in Rajasthan have PTB. In Rajasthan, there have been several PTB instances documented.

Dataset

The data collected from 1951 blood samples for the current study from six distinct Rajasthan districts: Dholpur, Bikaner, Jaipur, Udaipur, Jaisalmer and Dausa. 1,086 cattle and 865 goats made up the sample. Six districts in Rajasthan, roughly 15.06% of the total area covered, were the sites from which blood samples were taken.

Inclusion criteria

- Inclusion criteria comprised samples from cattle and goats, a total of 1951 blood samples, with a specific emphasis on apparently healthy animals.
- Blood samples from cattle and goats across six distinct districts in Rajasthan.

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- The geographic scope encompassed Bikaner, Dausa, Dholpur, Jaipur, Jaisalmer and Udaipur, representing approximately 11.06% of the total area covered in the study.

Exclusion criteria

- Restrictions on usage were essential to refine the dataset. The focus was kept on specific species by excluding non-goat and non-cattle species.
- Clinically unwell animals were excluded to uphold the study's emphasis on healthy subjects.

RESULT

In this section, we discuss the analysis of PTB seroprevalence in goats and cattle. A total of 1951 blood samples were drawn from goats and cattle from animal farms located in the Rajasthan districts of Udaipur, Bikaner, Dausa, Dholpur, Jaipur and Jaisalmer. Animals suspected of having PTB were given blood samples if they displayed symptoms such as edema, weight loss and diarrhea in the pipestream, which is caused by hypoproteinemia from protein-losing enteropathy along with decreased milk production. Blood was drawn from an animal that seemed to be in good health. Epi Info software was utilized to do statistical analysis on the data of these districts.

Data analysis

Blood samples must be carefully prepared for the ELISA test to guarantee the extraction of pertinent components for precise analysis. Using aseptic methods, vacutainers or syringes are used to draw blood samples from subjects while taking the ELISA assay's particular needs into account. If the blood is serum, it is allowed to coagulate before the serum or plasma is separated from the cellular components by centrifugation.Transferring the separated serum or plasma to a clean tube is essential to prevent contamination. Concentrations are measured using a micro-plate reader after colour development and the ELISA kit's standard curves or pre-established standards are used to determine the concentrations. Figure (1) presents the serum separated from blood.

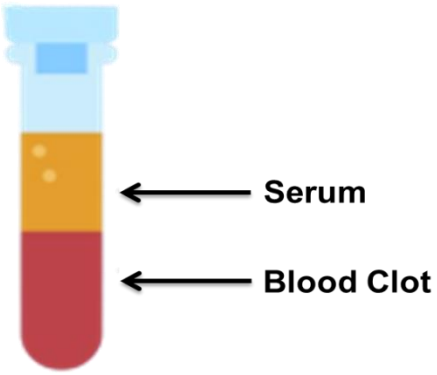

Figure (1). Serum separated from Blood

(Source: https://www.h-h-c.com/serum-or-plasma-what-are-their-uses-and-which-is-better-for-research-purposes/)

ELISA for detection of antibodies against MAP

Antibodies against MAP can be found in blood samples from goats and cattle using the commonly used ELISA technique. By immobilizing certain MAP antigens on microtiter plates and adding animal blood serum, this diagnostic method enables any antibodies to attach to the immobilized antigens. An enzyme-linked secondary antibody is added after the stages of washing to get rid of unbound components. A reaction that results in a

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detectable signal, such as a colour shift, is started by the subsequent addition of a substrate. There will be a MAP infection present because the strength of this signal is correlated with the quantity of antibodies present. The necessity of additional confirmation by complementary tests in the event of positive ELISA findings highlights the significance of a thorough diagnostic strategy in the management of PTB in cattle. Table (1) depicts the ELISA test findings demonstrating the existence of MAP-specific antibodies.

Table (1). The ELISA outcomes of tests demonstrating the existence of MAP-specific antibodies (Source: Author)

City	Total number of ELISA-tested samples	Positive ELISA results	Negative ELISA results	% the age of positivity
Bikaner	480	14	466	2.91%
Dausa	385	35	350	9.09%
Dholpur	265	3	262	1.13%
Jaipur	256	4	252	1.56%
Jaisalmer	270	1	269	0.37%
Udaipur	295	2	293	0.67%
Total	1951	59	1892	3.02%

Considering that the two districts in which positive samples were discovered are Dausa and Bikaner. Therefore, using the statistical analysis carried out using the program, additional data analysis was limited to the data of these two districts. Based on the odds ratio, the district of Dausahad a 6.18 times greater probability of PTB incidence than the district of Bikaner ($p \leq 0.05$). There was no discernible correlation found between the disease's prevalence and age, farmer education, milk output, or animal kind (cattle or goat). Figure (2) as well as Table (2) shows the prevalence of PTB in Dausa and Bikaner districts.

Table (2). PTB prevalence in Dausa and Bikaner districts (Source: Author)

City	Tested	positive	Prevalence
Bikaner	480	14	2.91%
Dausa	385	35	9.09%
Combined	865	49	5.66%

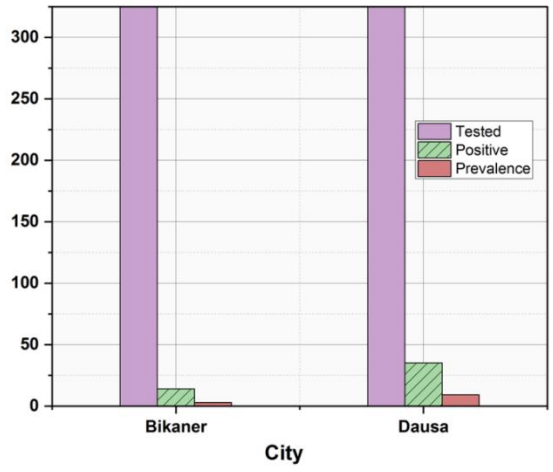

Figure (2). Result of PTB prevalence in Dausa and Bikaner districts (Source: Author)

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CONCLUSION

The seroprevalence of PTB in goats and cattle is demonstrated by the present research. An ELISA was used to examine the blood sample taken from Rajasthan (Udaipur, Bikaner, Dausa, Dholpur, Jaipur and Jaisalmer) using serum that was taken from the blood of these areas' animals. It was discovered that animals tested positive for PTB in two Rajasthan cities, Bikaner and Dausa. The findings revealed that PTB seroprevalence was 2.91% in Bikaner and 9.09% in Dausa and their combined prevalence was 5.66%. No positive samples were found in Dholpur or Jaipur. There was no expected correlation found between the disease's prevalence and age, farmer education, blood output, or animal species (goats or cattle). The specimen of blood could have been contaminated for a number of causes. The illness could have spread by pseudo-vertical transfer or through vertical transmission. In the future, examine the effectiveness of current vaccinations as well as create an innovative vaccination plans for cattle and goats against PTB.

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