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Sero-Prevalence of Paratuberculosis in Cattle and Buffalo Population at Abattoir in district Jhang, Pakistan

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ABSTRACT

Paratuberculosis is an important disease in the dairy industry that adversely affects the production of dairy animals. It affects all domestic animals, including cattle, sheep, goat, camels, and wild ruminants. To detect the prevalence of this disease locally, blood and tissue samples were collected randomly from cattle and buffalo, which were clinically weak and healthy animals. These samples were also collected from the abattoirs of district Jhang. Gross evaluation and grading of tissue samples were done based on thickening and severity of corrugations of intestinal tissue samples. Histopathological grading of tissue samples was done through H&E staining. Serum evaluation was done by checking the values of total protein and albumin in serum. Indirect ELISA was done to determine the seroprevalence of Paratuberculosis locally. A total of 140 tissue samples (n=70 from cattle and buffalo each) were evaluated for gross pathological examination. Based on gross pathological examination, 25% tissue samples showed no lesion with 18% of mild, 18% moderate, 17 % severe intensity levels. The physically weak and diarrheic animals were observed with lower mean values of total protein and albumin. A higher albumin mean value ($3.47_{\pm}$ 0.11 g/dl) was observed in buffalo, while the lower albumin mean value ($3.00_{\pm}$ 0.06 g/dl) was recorded in cattle. Histopathological grading of 140 tissue samples were evaluated based on the presence of microphage infiltration, epithelioid cell infiltration and granulomatous reaction. In grade I, 70 intestinal tissue samples were positive with the presence of a few macrophage infiltration or epithelial cells in lamina propria of villi and between crypts and payer patches. In grade II lesions, 40 tissue samples exhibited moderate macrophages of infiltration or epithelial cells in lamina propria of villi and between crypts. A total of 40 tissue samples were included in the grade. In grade III, 30 tissue samples showed granulomatous enteritis with an abundant number of macrophages. In various tissue samples, payer patch hyperplasia was prominent. A total of 100 sera samples of cattle and 40 buffalo were screened through ELISA. A total of 6/140 sera samples were found positive in cattle and buffalo with a 4.2% prevalence in slaughterhouses of Jhang based on the ELISA test.

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INTRODUCTION

Pakistan dairy industry is at 4th number in milk production throughout the world and among various infections paratuberculosis is a serious threat for dairy industry because it effects the production of dairy animals. It affects all the domestic animals which include cattle, sheep, goat, camels and wild ruminants (Munir et al. 2014). Large populations of small and large ruminants are affected by this disease with an estimated annual economic loss of 200-250 million USD in the US dairy sector (Berget and Ginn 2000).

This disease is caused by acid fast bacillus *Mycobacterium avium* sub spp. *paratuberculosis* (MAP) which does not give response to heat and cool and many disinfectants (Whittington and Sergeant 2001). Johne and Fort Hingham had reported this organism in 1895 (Kumar et al. 2017). This infection agent does not make spore, they are non-motile, branched, slightly curved and rod shapes. This organism is found in water and soil (Whittington et al. 2005). Granulomatous enteritis is caused by this organism due to which width of intestinal wall increase and absorption of food does not occur which eventually become cause of death (Nielsen et al. 2008). This organism also causes irritable bowel syndrome in human beings (Sechi et al. 2001). MAP resides in macrophages, which is phagocytized by macrophages and mainly accumulate in lamina propria of intestine due to which mucosa of intestine become thickened and corrugated (Sivakumar et al. 2006). Basic Histopatho-logical changes which were mostly observed in affected intestines are three, lepromatous lesion, tuberculoid lesion and infiltration of giant cells in lamina propria mostly observed (Brady et al. 2008). After entry of this organism in animal body, animal does not appear clinical signs but shed this organism in feces and milk (Munir et al. 2014). This organism could be evaluated in milk and dairy products because it showed resistance to the cheese making process and pasteurization. The ingestion of organism through colostrum was the main cause of transmission in calves but they develop clinical signs in adult life due to its prolonged incubation period (Kurade et al. 2004).

MAP prevalence reported in dairy herd of Spain was 4.03% (Diéguez et al. 2007). In dairy herds of India Map presence was 2.71 % (Singh et al. 2008). In Pakistan, the prevalence of Paratuberculosis in sheep and goat was recorded up to 9% and 5% at slaughterhouse (Chaudhry et al. 2009). Another study revealed the overall prevalence of Paratuberculosis at 4 livestock farms of public sector in Punjab in water buffalo as 1.3% (Rehman et al. 2018)

This data suggests that MAP infection is present in all over the world not only in Pakistan. Based on its economic and public health significance this study was designed on paratuberculosis in cattle and buffalo which come at abattoir Jhang District. Our goal of study was to check out the seroprevalence of paratuberculosis in cattle and buffalo population at Slaughterhouses of Jhang District.

MATERIALS AND METHODS

Sampling procedure

Samples were collected randomly from the Govt. slaughterhouses of District Jhang of Punjab province. Both healthy and emaciated animals were selected for sample collection. After removal of skin, intestine was exposed carefully and spread on floor. Thickened intestinal tissue samples were observed, especially between ileum and ceca junction with adjacent mesenteric lymph nodes for further investigation. Sample from affected intestine and lymph nodes (n=140) was collected and preserved into 10% NBF from June to October 2019.

Gross lesion

Tissue samples were collected from intestine where corrugation was prominent. The thickening of the intestine wall was 2 to 5 times thicker than normal. Intestinal tissues were categorized on the basis of thickness and severity of corrugations. Mucosa was shown brain like corrugations which is not flattened on stretching. Mesenteric lymph nodes were swollen and juicy.

Histopathological studies

A total of 140 sections from intestinal part of ileum were stained (H and E) and adjacent lymph nodes were observed under microscope 4x, 10x, and 40x objectives. The lesions were classified into different grades, depending on the quantity and type of cellular infiltrate (lymphocyte, epithelial cells and macrophages). Lesion scoring of tissue sections was done through H&E staining according to the standard protocol of (Bancroft and Gamble 2008).

Biochemical Studies

Blood sample was taken from diseased cattle for serum biochemistry. The blood samples were coagulated by standing tubes vertically at room temperature. Then these samples were centrifuged at 1500 rpm for 10 mints. Supernatants were stored in 1ml aliquots at -80°C till use (Piras et al. 2014). Total protein and albumen were evaluated through auto-analyzer (Optizen) using commercial kits (El-Deeb et al. 2014).

Total protein and albumen were determined by using the commercial kits (ITRON) following the protocol mentioned on broacher. For albumen evaluation, the values were adjusted following the manufacturer's instructions in terms of wavelength 630 nm, temp. 20-37°C, reaction type end point. All the serum samples were kept under room temperature for melting. For albumen calculation in serum samples, the standard reagent was run first to calculate its value. Both the reagent (1000 µl) and sample (10 µl) were mixed together in a test tube followed by incubation (5 mins). The samples in test tube containing the reagent were run.

Serum Analysis

Blood samples were collected in the tubes without anticoagulant. Blood samples were processed parallel to the tissue samples. The blood samples were allowed to clot at room temperature followed by centrifuged at 3000-5000 rpm at 18°C. Serum samples were carefully transferred to the micro tubes and stored in aliquots at -20 °C until used for ELISA test (Abdulrasool and Mahdi 2016). The ELISA was performed following the method described in commercial kit (ADIVET, France Ruminant serum paratuberculosis Advanced kit). The extra

conjugate was removed through washing step followed by addition of substrate link of enzyme to complex and transferred into color product. The absence or presence of antibodies was determined using threshold values obtained from positive control. The comparison of their optical density (OD) value, the presence or absence of antibodies against MAP was determined.

Data Analysis

All data of serum chemistry was presented as mean ± SE of mean by using student *t*-test.

RESULTS

Gross Lesions

In our present study, we found many intestinal tissues of cattle and buffalo with thickened and corrugated mucosa (Table 1). These lesions were extending from duodenum to rectum longitudinal and transverse corrugations were present having the irregular folds (Fig. 1).

Table 1: Gross lesions of different tissues showing intensity and lesion score.

Tissues	Gross lesions	Intensity of Lesion	Lesion score
Intestine Ileum, Cecum (Icj)	1.Thickening	No lesion	0
		Mild	+
		Moderate	+ +
		Severe	+ + +
	2.corrugations	No lesion	0
		Mild	+
		Moderate	+ +
		Severe	+ + +

Histopathological Evaluation

A total of 140 sections from the intestinal part of ileum were stained (H and E) and adjacent lymph nodes were observed under microscope 4x,10x, and 40x objectives. Lesions were classified into different grades, depending on the amount and type of cellular infiltrate (lymphocyte, epithelial cells and macrophages) (Fig. 2A and 2E).

In grade, In intestinal tissues were considered positive when there was few macrophages infiltration or epithelial cells were clearly present in lamina propria of villi and between crypts and there were payer patches. A total of 70 tissue samples were included in the category (Fig. 2B).

The Grade II lesions, there was moderate macrophage infiltration or epithelial cells clearly present in lamina propria of villi and between crypts. A total of 40 tissue samples were included in grade (Fig. 2D).

Grade III lesion, there was granulomatous enteritis with an abundant number of macrophages. In various tissues, payer patch hyperplasia was prominent. The villi showed different changes, villous distortion and thickening by inflammation of cell infiltration, fusion of villi, and atrophy. A total of 30 tissue samples were included in this grade (Fig. 2C).

Biochemical Analysis

Mostly cattle that came to slaughterhouses were physically weak and had diarrhea. Cattle which were physically weak and had diarrhea. Their intestines were swollen and corrugated mucosa showed lower mean value of total protein such as cattle had lower mean value 6.66total protein and respectively albumin mean value was also lower 3.00 (Table 2). These results are significantly different at $p< 0.05$. While most buffalo that came to slaughterhouses were not with diarrhea and showed higher mean values 7.38of total protein and higher mean value of Albumin 3.47 were noted.

Biochemical analysis findings revealed significant decrease in total protein and albumin values in those animals which were physically emaciated. They had diarrhea and their intestines were severally corrugated.

Serological Analysis

A total number of 140 adult cattle and buffalo above the age of five years were screened for paratuberculosis based on expected prevalence in our study. For calculation of sample size 95 % confidence interval relevant formula was used with 10% expected prevalence with 5%absolute precision. n=1.962Pexp (1-pexp)/d2.

The result revealed that 6/140 samples were found positive in cattle and buffalo, with a 4.2% prevalence in Jhang slaughterhouses, Based on the ELISA test. A total of 100 cattle samples and 40 buffalo samples were screened through ELISA (Table 3, Fig. 3).

Fig. 1: Longitudinal (A) and transverse (B) sections of intestinal tissue corrugations/lymph nodes with blackish medullary region.

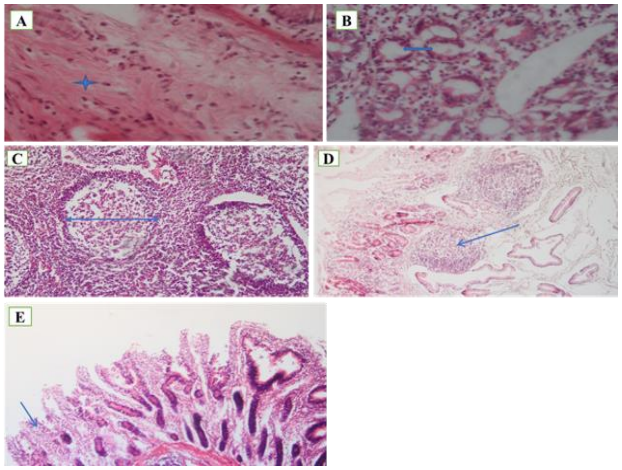

Fig. 2: Macrophages showing vacuolated cytoplasm appearance and presence of fibrous connective tissue (FCT) deposition predominantly collagen fibers along with inflammatory cells (H&E;400x). **B.** Necrotic changes shown in Crypt walls with loss of epithelium (Thin arrow) and presence of inflammatory cells masses among crypts. Vacuolation (double arrow) in epithelial cells of Crypts seen clearly. **C.** Enlarged lymphoid follicle with light-stained germinal center indicates proliferation of lymphoid masses in cortical region of lymph node. **D.** Proliferation of Peyer's patches seen (Thin arrow) inside intestine (H&E; 200x). **E.** Surface epithelium showing erosions & necrotic changes inside epithelium (H&E; 200x).

Table 2: Protein profile of cattle and buffalo with Albumin and Globulin contents variation.

Animal	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Cattle	$6.66^{a} \pm 0.18$	$3.00^{a} \pm 0.06$	$3.60^{a} \pm 0.16$
Buffalo	$7.38^{b} \pm 0.20$	$3.47^{b} \pm 0.11$	$3.90^{a} \pm 0.14$
P-Value	0.00	0.000	0.16

Table 3: Indirect ELISA results

Parameters	Positive/ Negative	Positive%	Chi square value
Total number of animals	6/140	4.2%	
Species			
Cattle	3/100	3%	
Buffalo	3/40	7.5%	
Age			
7year	1/45	2.1%	17.857^{a}
<7year	5/95	5.2%	
Sex			
Male	1/60	1.6%	17.857^{a}
Female	5/80	6.25%	

DISCUSSION

In this study, the pathological lesions of intestinal tissues and the seroprevalence of Johne's disease were evaluated in healthy appearance and weak buffaloes and cattle by using different diagnostic tests, histopathology, serum chemistry, and ELISA. In gross lesions, mucosa of the intestine was corrugated and thickened in cattle and buffalo tissue samples. The serum chemistry evaluation also proved severe intestinal corrugation and thickening of animals with low total protein and albumen values. Serum

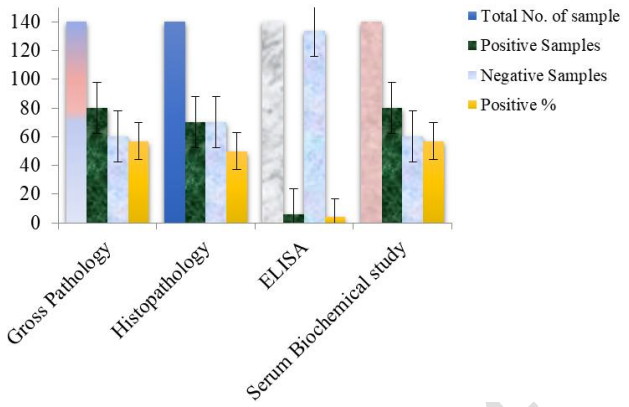

Fig. 3: Comparative relationship between different diagnostic techniques.

and intestinal tissue were collected from weak animals that had suffered from a problem of shooting diarrhea. The present study showed a prevalence of 4.2% in cattle and buffalo on basis of ELISA. These results for the prevalence of bovine paratuberculosis based on ELISA were similar to those of previous study that reported its prevalence of 3.8% in both cattle and buffalo based on ELISA and purified protein derivative (PPD) at a dairy farm (Sikandar et al. 2012). In Faisalabad, an overall prevalence of Paratuberculosis was recorded up to 31.8% and 37.8% in cattle and buffalo (Ahmed et al. 2019). The difference in prevalence rate was expected due to culling and slaughtering of low-productive and untreated animals at abattoirs. The untreated and chronic stage of this disease adversely affects the production, thus leading to culling animals (Arrazuría et al. 2014). The prevalence of paratuberculosis is higher in dairy cattle (54.1%) than beef cattle (<5%) (Ott et al. 1999). ELISA based seroprevalence of Jhone's disease in India was reported up to 15.4% while in Iran its seroprevalence reported up to 4.34% (Pourmahdi Borujeni et al. 2021). The results of present study are like that of seroprevalence paratuberculosis in Iran which exhibit its persistence in district Jhang.

In the current study, albumin and total proteins contents were significantly decreased in animals with enteropathy and diarrhea. This infection enteropathy leads to intestinal malabsorption (Sweeney 2011). The hypoproteinemia may be due to decreased albumin content associated with leakage of albumin from damaged tissues. These changes were reported in infected cattle with paratuberculosis suffering from diarrhea. This study manifests the same results in physically weak, emaciated and diarrheic cattle and buffaloes are hypoproteinemia and remain suspected for paratuberculosis. Because both hematological and biochemical studies were most used for the diagnosis of Jhone's disease in camels and cattle (Elmagzoub et al. 2020; Salem et al. 2019).

In the current study, paratuberculosis is classified into three different types based on histopathological lesions. Some intestinal tissues were considered positive with few macrophage infiltration or epithelial cells present in lamina propria of villi and between crypts and payer patches. There was moderate macrophage infiltration or epithelial cells were present in lamina propria of villi and between crypts in some intestinal tissues. Granulomatous

enteritis with an abundant number of macrophages was observed in most intestinal tissues. In various tissues, payer patch hyperplasia was prominent. The villi showed various pathological changes including villous distortion and thickening by inflammation of cell infiltration, fusion of villi, and atrophy. In classification, epithelioid were not encountered, and lymphocyte infiltration was associated with lesions. Mainly the intestinal tissues were classified based upon the presence of macrophages. Multifocal granuloma replaced the cortex and medullary in mesenteric lymph nodes, and macrophages are filled with Kerry hectic debris of apoptotic lymphocyte (Koul et al. 2004; Weiss and Souza 2008). Previously, it has been shown that macrophages infected with mycobacterium tend to activate apoptotic pathways. In the case of lymph nodes, there was the presence of lymphocytes and prominent macrophages along with immature granulomatous reactions with inflammatory cells.

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