ABSTRACT

Bubaline paratuberculosis is a chronic, debilitating disease that has been implicated in causing reduced milk yield and poor carcass quality in affected animals. The objective of the current study was to depict the histopathological manifestations of paratuberculosis in subclinically infected Nili-Ravi buffaloes. For this purpose a total of 53 slaughtered carcasses were selected on the basis of thickened/ corrugated intestinal mucosae along with edema of the associated lymph nodes. Acid-fast bacilli (AFB) were observed only in 22.64% of the cases on smear method examination. Histological examination of the tissue samples was performed using hematoxylin-eosin and Ziehl-Neelsen staining methods. Heavy mononuclear cells infiltration was detected in the tissues. The foamy cytoplasm of epithelioid-macrophages was observed to be engorged with AFB in the positive cases. The results of this study demonstrated tissue sections showed much higher positive results (100%) as compared to the smear method (18.68%). Furthermore, the overall histological representation of tissue sections was more defined and illustrative than their smears. The histologically positive cases were also confirmed by means of ELISA testing. Despite being a time-honored technique, histopathology is an incredibly accurate and reliable protocol for diagnosing bubaline paratuberculosis.

Keywords: Nili-Ravi buffaloes, paratuberculosis, staining, histology, ELISA

INTRODUCTION

Recently the paratuberculosis (PTB) has become one of the most important livestock diseases. It is highly infectious and incurable in nature (Jones et al., 2006) and has been documented in domestic animals throughout the world (Buergelt and Ginn, 2000). Also, it has been observed in zoo and wild ruminants (Ayele et al., 2001; Erume et al., 2001). The causative agent of PTB is an acid fast gram positive bacillus, termed as Mycobacterium avium subsp. paratuberculosis (MAP) (Antognoli et al., 2007). Young animals are believed to be highly susceptible to PTB infection (Collins, 2003). Two distinct forms of PTB have been distinguished in animals as paucibacillary and multibacillary forms.
Crohn’s disease (CD) in humans is also suspected to be caused by MAP (Erume et al., 2001). Subclinically infected animals constitute the most significant source of infection and accurate diagnosis of these animals is critical for the control of PTB. Among large number of laboratory tests, the histology of intestine and mesenteric lymph nodes is the most suitable trial for the diagnosis of PTB (Buergelt and Ginn, 2000; Maxie et al., 2007). Although culture technique is regarded as the gold standard (Huntley et al., 2005) nevertheless, it is a labor intensive technique and requires a prolonged incubation period of up to 16 weeks. The current study was devised to investigate various histological changes in the gut (intestine) and mesenteric lymph nodes and to compare its diagnostic performance with Ziehl-Neelsen (ZN) stained smears and ELISA technique.

**MATERIALS AND METHODS**

**Samples**

A total of 53 tissue (intestine and mesenteric lymphnodes) samples were collected from animals suspected of having PTB for investigation from two abattoirs in the Municipal Area of Jhang, Pakistan. The majority of the animals brought for slaughtering were weak and emaciated. Collection of blood (for serological evaluation) and tissue samples was performed before slaughtering and at postmortem, respectively. The collected samples were kept in sterile vaccutainers and plastic bags, respectively, that were subsequently transferred to a cooling jar containing ice. The tissue samples were conveyed to the histopathology laboratory, College of Veterinary and Animal Sciences, Jhang for further processing. Whereas serological evaluation was conducted at the University Diagnostic Laboratory (UDL), University of Veterinary and Animal Sciences, Lahore, Pakistan.

**Screening through ZN smears technique**

The thickened portion of intestine having prominent mucosal corrugations was used for the preparation of a few hardpressed mucosal impression smears. The ZN staining method proposed by Cappuccino and Sherman (2008) was adopted for staining of intestinal impression smears (ISs). Acid-fast bacilli (AFB) were observed as reddish rod-shaped structures under the microscope. Those samples in which the ISs were ZN stain negative were all discarded and all those that showed AFB were further processed for histopathological examination following Bancroft and Cook (2007). ZN staining of the tissue samples was performed using the method proposed by Huntley et al. (2005). Serum samples of the ZN positive animals were subjected to an indirect ELISA test. The indirect immunoenzymatic technique was executed using the diagnostic kit of anti-MAP antibodies in bovine sera as per instructions of the manufacturers (SerelisaTM M.Para TB Ab Mono Indirect, Cat. No. ASPTB3 (2 Plates), Symbiotic Europe SAS, 2, rue Alexander Fleming, F-69367 Lyon, Cedex 07, France). Data thus collected for ZN acid-fast staining (smears as well as tissue sections) and the ELISA test were analyzed by applying descriptive statistical methods (Zar, 2003).

**RESULTS AND DISCUSSION**

A total of 53 samples obtained from Nili-Ravi buffaloes suspected of having PTB were tested. All the specimens illustrated relatively thickened and extremely corrugated mucosae more around the distal ileum; however, the gross pathological
changes were evident in smear samples that were positive for AFB. The diverse elevated corrugations of the thickest parts were prominent enough to retain their appearance even after firm stretching. However, mesenteric lymph nodes of only nine animals were swollen and enlarged.

3.1 Histopathological examination of tissue sections

Overall 22.64% (12/53) mucosal smears from intestines were found to be positive for ZN stain. Taking them as the confirmed PTB cases, further investigation of their impression smears and tissues sections was conducted. Intestine and mesenteric lymph nodes were the organs of study. Histological findings reflected that all (100%) of the (12/12) intestinal sections were ZN stain positive. It is, therefore, clear that tissue section is a relatively superior technique for the diagnosis of PTB as compared to the smear method. A significantly greater number of AFB was observed in intestinal tissue sections as compared to intestinal ISs. Likewise, the number of positive detected samples was also higher through histological sections of mesenteric lymph nodes contrary to its ISs. Histopathological studies revealed that the epithelial cells lining the intestinal villi had mostly sloughed-off in the posterior small intestine, and the mucosal lamina propria was fully distended with granulomatous infiltrations. The predominant cells of the infiltrate were the epithelioid macrophages having foamy cytoplasm and round to oval, eccentric nuclei (Figure 1). The intestinal walls were thickened up to twice normal owing to increase in the size of mucosa and submucosa. Fibrous connective cells along with lymphocytes, plasma cells and eosinophils were extensively infiltrated in the mucosa and submucosa. Crypts of Lieberkühn had become shallow, slit-like, atrophied, reduced in numbers and were separated from each other due to cellular infiltration. Exfoliated cells were observed in the opening of the crypts. A few infiltrations by mononuclear cells in the tunica muscularis and even in serosa were also evident. While using ZN stain, the cytoplasm of epithelioid macrophages was stuffed with acid-fast microorganisms. The AFB appeared red to pink inside macrophages (Figure 2). Large collections of laden macrophages were observed in the mucosa and submucosa. Multinucleated giant cells were also noticeable. Examination of all (n=09) oedematous lymph node samples revealed that their covering fibrous connective tissue capsules were very thick. There were variable sized areas containing shapeless, acellular brown caseous material surrounded by thin fibrous membrane. All the histologically positive cases were also confirmed by the indirect ELISA test.

The study was aimed to detect PTB in Nili-Ravi buffaloes via traditional methods i.e. ZN staining, histopathology and indirect ELISA. Emphasis was laid on the histological examination of intestine and mesenteric lymph nodes. Grossly observable gut lesions in small domestic ruminants are often mild (Radostits et al., 2007), but in the recent study the intestines were about two to three times thicker than normal and presented conspicuous transverse corrugations the same as reported by Sikandar et al. (2012). The thickness and corrugations occurred as a result of cellular infiltration in the mucosa and submucosa as described by Ayele et al. (2001; Jones et al. (2006); Sivakumar et al. (2006); Khan et al. (2010); and Sikandar et al. (2013). Intestinal tissue contained multinucleated giant cells. However, our results do not comply with those of Tafti and Rashidi (2000), who described giant cells inside mesenteric lymph nodes in goats. This study highlighted the
Figure 1. Mucosa of the small intestine. Epithelioid macrophages having foamy cytoplasm and eccentric nuclei are seen in the lamina propria. H&E (400X).

Figure 2. Mucosa of the large intestine. Macrophages laden with rose red acid-fast bacilli. Crypts are present wide apart from each other in upper portion of mucosa of the cecum. ZN (100X).
validity of a thorough histological assessment of the intestine and mesenteric lymph nodes for the identification of PTB in one of the most important dairy animals. The impression smear method used alone to diagnose PTB is not reliable unless verified by the histopathological method. No doubt the histological method is a relatively old and time consuming approach, but it is one of the most economical techniques and provides high specificity and accuracy as compared to other conventionally used methods. It can be regarded as a routine diagnostic technique for PTB infection in buffaloes. Further study for the identification and differentiation of MAP is required, which is not probable using staining techniques. According to Ayele et al. (2001) and Shiratsuchi et al. (2008) MAP has been suspected to be linked to Crohn’s disease (human chronic granulomatous ileocolitis), and the existing means are inadequate to avert the access of MAP infected tissues to the food supply (Antognoli et al., 2007). Sufficient hygienic and public health measures should be undertaken to overcome this problem. Owing to the availability of limited information, it is not regarded as a priority disease for control in Pakistan. It can be concluded from this study that PTB is prevalent in Pakistan and precise diagnosis is possible using histological and serological techniques.

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REFERENCES


