

Pathological and molecular detection of diseases of cattle at slaughter

A. A. Jahan¹, T. Ruba¹, T. T. Mumu¹, M. S. Rana², M. Islam¹, S. M. S. H. Belal¹, M. A. H. N. A. Khan^{*1}, A. M. Bari¹

¹Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, ²Upazila Livestock Office, Mohanpur, Rajshahi-6220, Bangladesh

Abstract

Background

This study was carried out to identify important zoonotic diseases of beef cattle at slaughter in Bangladesh.

Methods

A total of 20 slaughtered cattle of both sexes, different ages and breeds were taken under investigation from Nilphamari, Rajshahi and Mymensingh district, Bangladesh during July to October, 2017. Detailed ante-mortem and post-slaughter lesions were inspected and the tissues from the lungs, liver, kidney and spleen were collected aseptically for histopathological examination. The tissue sections were processed and stained with hematoxylin and eosin (H&E) stain, acid fast stain and Gram stain. To detect the presence of specific microorganism in the tissues, the genomic DNA was extracted and polymerase chain reaction (PCR) was carried out.

Results

Grossly, liver appeared as most affected organ followed by lungs, kidney and spleen. Wide spread necrosis and pipe steam liver due to fascioliasis (n=4), hydatidosis in lungs (n=3), congestion in the kidney (n=4) and petechial hemorrhage (n=2) in the spleen were the predominant lesions observed in the slaughtered cattle. H&E staining of tissue sections revealed granulomas in two liver and one lungs. Lung section stained with acid fast stain detected the acid fast bacilli in a case. Tissue sections stained with Gram stain showed cocci bacteria in the liver of two cattle. The PCR confirmed the presence of *Mycobacterium tuberculosis* in a cattle.

Conclusions

Fascioliasis, hydatidosis and important zoonotic disease like tuberculosis are prevalent in the slaughtered cattle in Bangladesh. PCR technology appeared as a sensitive and specific screening test to diagnose tuberculosis. However, screening of large number of samples is necessary to detect the presence of important zoonotic diseases in cattle for safe beef production.

Key words: Cattle; Zoonotic Diseases; Slaughter houses; PCR; Tuberculosis

*Correspondence: hadi.khan@bau.edu.bd

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Introduction

Cattle production is an essential phenomenon of our livestock economy in Bangladesh in terms of supplier of meat, milk, draught, hides, bone and other derivatives (Anon, 2008). Due to lack of awareness of meat-borne zoonoses the life of livestock producers, abattoir workers and the general public are at risk of infection (Maxwell, 2005; Ahmedullah *et al.*, 2007; Mellau *et al.*, 2010; Alawa *et al.*, 2011). Records of ante-mortem and post-mortem inspections are useful epidemiological data for the evaluation of diseases at farm level and to verify the efficacy of prophylactic and therapeutic interventions (Ogunrinde, 1980; Antia, 1982). In this regards, slaughter houses can provide an excellent opportunity for detecting pathological lesions of both economic and public health importance (Vecerck *et al.*, 2003; Raji *et al.*, 2010). Frequent abattoir surveys have been conducted in different countries to detect the gross and microscopic lesions in lungs, liver, spleen and kidney of cattle (El-Dakhly *et al.*, 2007; Mwabonimana, 2008; Belkhirri *et al.*, 2009; Raji *et al.*, 2010; Alawa *et al.*, 2011). However, the gross and microscopic lesions usually indicate the advanced stages of diseases. Therefore, along with the pathological findings, the use of specific laboratory tests and isolation and molecular detection of the infecting agents is necessary (Corner, 1994; Shitaye *et al.* 2006).

The daily encounters of diseases and the concern of the veterinary public health staff at abattoir convinced us to carry out a survey of diseases associated with slaughtered cattle at abattoir. It is necessary to know the extent to which the people are exposed to zoonotic diseases associated with abattoirs. But, little is known on the economic and public health aspects of carcass condemnation in Bangladesh. Few studies have been conducted in this regards and the frequently detected abattoir diseases or conditions were fascioliasis, taeniasis and hydatidosis (Affroze *et al.*, 2013; Salmo *et al.*, 2014; Islam *et al.*, 2015). This study involved gross and histopathologic investigation of lungs, liver, kidney and spleen as well as polymerase chain reaction detection of important zoonotic diseases of cattle at slaughter.

Materials and Methods

Collection of sample

This investigation was carried out on randomly selected 20 slaughtered cattle of different ages, sexes and breeds from different slaughter houses of Nilphamari, Rajshahi and Mymensingh district. In every case, a detail ante-mortem and postmortem examination was performed to identify the gross pathological changes in lungs, liver, kidney and spleen. Tissue samples (lungs, liver, kidney and spleen) for histopathological examination were collected in 10% neutral buffered formalin and transported to the Department of Pathology, Bangladesh Agricultural University, Mymensingh. Tissues from lungs, liver, kidney and spleen were also collected in sterile tubes and stored at -20 °C for molecular detection of specific diseases. The formalin fixed tissues were processed, sectioned and stained with hematoxylin and eosin (H&E) stain, Gram stain and acid-fast stain (Luna, 1968).

Extraction of Genomic DNA

DNA was extracted by locally adopted conventional method from representative tissue samples of slaughtered cattle (Buckingham and Flaws, 2007). In brief, a total of 20 mg tissues sample was crushed in pestle and mortar in liquid nitrogen, crushed tissues was taken in microcentrifuge tube containing 600 µl of cell lysis buffer and vortexed. The solution was centrifuged at 5000g for 10 mins to collect the supernatant. Equal volume of phenol chloroform isoamyl (PCI) alcohol was added to the supernatant and vortexed. Then the solution was centrifuged at 10000g for 2 mins and the supernatant was collected. Thereafter 10µl of 5M NaCl was added on the 90 µl solution. Ice cool absolute alcohol of 250 µl was added, centrifuged at 13000g for 15 mins and the supernatant was collected. The supernatant was desalted twice by using 80% ethanol. The tube was allowed to air dry for 15 mins and 50 µl of nuclease free water was added to the tube and stored at 4°C. The concentration of genomic DNA achieved was between 50-100ng/µl in nuclease free and deionized distilled water. The purity and concentration of genomic DNA was measured in spectrophotometry (260°A/280°A) and agarose

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gel (1.5%) electrophoresis respectively to amplify genetic fragments of tuberculosis, (Spectronic R Genetics™ New York, USA). toxoplasmosis, brucellosis and leptospirosis (Table 1).

Selection of primer for molecular detection of diseases

Oligonucleotide primer sequences for PCR were designed by selecting genes from NCBI data base

Table 1. Primers and their sequences designed and used in this study

Primers	Sequences (5'-3')	Gene/Amplicon Size (bp)	Gen bank References
MTb1F1 MTb1R1	CGGTTTCAGGCTTCACCACAGTG GTGGTGTCTGGCGATGTGGTAT	H37Rv/ 346	AL123456.3
BrucF1 BrucR1	GATGGACGAAACCCATGAATG CGATGCCATCTTGTGGATGCC	alkB/ 621	AJ314591.1
ToxoplsF1 ToxoplsR1	GAATTCGTTTCGACAGAAAGGG CATTTCACCTGTATTTGCCAGC	B1/ 512	AF179871.1
LeptF1 LeptR1	CAATTCAAGACGCTGGAGTG GTGCCAAGACCAGGGATTC	HP/ 323	KP729624.1

Polymerase chain reaction (PCR)

PCR reactions were performed on each DNA sample in a 25 µl reaction mix containing 12.5 µl of 2X Master Mix (GeNei™ PCR Master Mix Kit), 1 µl (25 pmol) of each primer, 5.5 µl nuclease free H₂O and 5 µl template DNA/ water (for water control tube). DNA amplification was carried out in a thermal cycler (Master Cycler Gradient, Eppendorf, Germany) using the thermal profile: 95°C for 5 min, one cycle; 94°C for 30 sec, 66°C for 1 min, 65°C for 1 min, 68°C for 1 min, 67°C for 1 min, 35 cycles; 72°C for 9 min, one cycle. After completion of PCR reaction the tubes were held at 4°C.

Electrophoresis

The amplified PCR products were electrophoresed in 1.5% agarose gel, stained with ethidium bromide and examined under UV light using an image documentation system (Spectronic R Genetics™ New York, USA).

Results and Discussion

In this study, 80 tissue samples from 20 slaughtered cattle including lungs, liver, spleen and kidney were examined. The causal agents

targeted were *Mycobacterium*, *Brucella*, *Leptospira* and *Toxoplasma* species from the slaughter cattle of three cities. The H & E staining, acid fast staining, Gram's staining of tissue sections were carried out to aid in diagnosis. Further, the confirmation of the etiology of the diseases was attempted by using PCR technique.

In this study, the distribution of suspected lesions showing in the carcasses are shown in Table 2. The results indicated that the male cattle were affected more with various diseases than the female cattle; which could be due to the facts that they were not castrated, frequently move here and there, hunt for estrous females and spend little time to eat and drink. These may lead them hunger, catching more infections and disorders. Cattle aged between 3 to 6 years of age were the most vulnerable groups as they were infected more (Table 2). Naima et al. (2011) reported similar observation in her slaughter house survey and documented 3.58% incidence of tuberculous infection.

Table 2. Distribution of suspicious lesions in the carcass by age, sex and breed

Variables	n=20	Percentages (%)
Age		
≤ 3 years	2	10
3 – 6 years	11	55
6 – 9 years	4	20
< 9 years	3	15
Sex		
Male	13	65
Female	7	35
Breed		
Deshi	8	40
Cross	12	60

Gross lesions at necropsy in different organs are represented in the Table 3. It was found that the liver was the most affected organ as 50% (10 out of 20) of the examined cattle showed lesions in liver followed by lungs (30%, 6/20), kidney (20%, 4/20) and spleen (15%, 3/20). Among 10 livers with lesions 7 showed evidence of fascioliosis. This could be due to fact that fascioliosis is endemic in cattle in Bangladesh and cattle can be infected with fascioliosis at any time of their life (Karim *et al.*, 2015). Hydatidosis commonly infect lung tissues and hence lungs stand second in terms of infectivity of organs. Lesions in kidneys stand third, cattle mostly reared on pasture land from where they may take up dirty or rotten food staff, hence the toxic ingredient of food may pass through kidney and affected thereafter. The spleen is the sites where most of the infectious agents were destroyed but predominant lesions suggestive for specific infectivity was absent.

Table 3. Distribution of gross lesions based on their locations

Localization of lesions	n=20	Percentages (%)
Lungs	6	30
Liver	10	50
Spleen	3	15
Kidney	4	20

The spleen can be affected in clinically sick or sub-clinically infected cattle; noticeable hemorrhages were seen in the spleen of two cattle and these two cattle may have septicemia before death.

During necropsy, the study found nodular development with congestion and hemorrhages in lungs of one cattle (Fig. 1a). Bovine tuberculosis is usually characterized by the formation of granulomas (Singh *et al.*, 2017) and the studied animal was suspected to be a case of bovine tuberculosis. In cattle, multiple proliferative necrotic granulomas in the lungs, liver and nervous system is characteristic sign of bovine toxoplasmosis (Bhopale, 2003; Dubey and Jones, 2008). However, such lesions were not found in any of the studied animals. Cystic structure embedded in lungs tissues was seen in three cases. On incision, clear watery fluid was coming out and suspected as a case of hydatidosis. Pipe stem liver was seen in the liver of three cattle and the liver was severely necrosed (Fig. 2c). Presence of pipe stem liver indicates fascioliosis. Chronic fascioliasis may provide risk for tuberculosis (Flynn *et al.*, 2007) and the cattle showed tuberculous nodules also had pipe stem liver; a relation between fascioliasis and tuberculosis was interlinked. In this study petechial hemorrhage in the spleen was seen in two cases (Fig. 2a). There was hemorrhage in the upper respiratory tract and serosal surface indicating that the animal was suffering from septicemic disease immediately before slaughter. Petechial hemorrhages were common and may be extensive, and organs are often discolored due to icterus, such lesions may also be due to *Leptospira* infection (Levett, 2001) but was absent in this study.

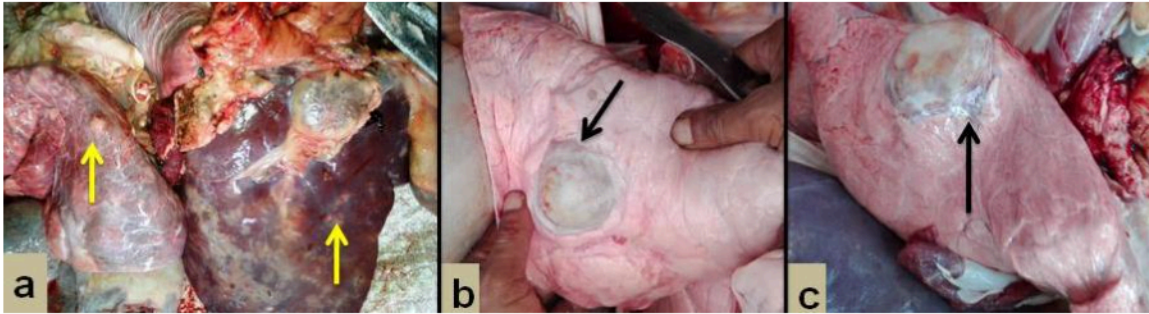


Figure 1: Examination of lungs of cattle at slaughter showed the presence of caseous necrosis (a, yellow arrow) and hydatid cysts (b and c, black arrow).

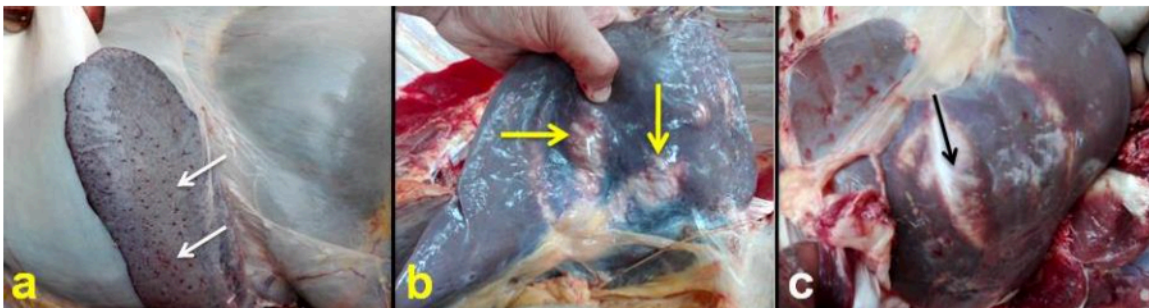


Figure 2: Examination of spleen and liver of slaughtered cattle. Petechial hemorrhages on spleen (a, white arrow), widespread necrosis of liver (b, yellow arrow) and pipe stem liver (c, black arrow) were seen.

However, at necropsy, most of the diseases could not be confirmed. Representative samples were, therefore, studied using histopathology. The histopathological examination of tissue sections stained with H & E showed granular and nodular inflammation and abscess formation in lungs (Fig. 3a, b). The granular lesions may be due to tuberculosis. Folia *et al.* (2016) reported accumulation of live and dead macrophages and other inflammatory cells in bovine tuberculosis. There were plenty of macrophages in the lungs parenchyma. Furthermore, lung tissue sections stained with acid fast stain showed acid fast bacilli in a case (Fig. 3c), and the case was suspected as Mycobacterial infection. This result supported the findings of Rhyan *et al.* (1995) and stated that acid-fast bacilli were rare to numerous

and were randomly scattered in the margin of caseous necrotic tissue. Inflammatory cells of tuberculosis infection were predominantly macrophages. In this study acid fast bacilli were seen in macrophages and inflamed tissues of lung. The routine H & E staining and acid fast staining of tissue sections were further aided by using Gram staining method. Tissue sections obtained from the cattle with abscesses formation showed plenty of Gram positive bacteria in the inflammatory zone and they were cocci in appearance (Fig. 4a, b, c). The species of incriminated bacteria was not confirmed, however, the bacterial species involved in abscesses formation may be either Streptococci or Staphylococci that will require further investigation.

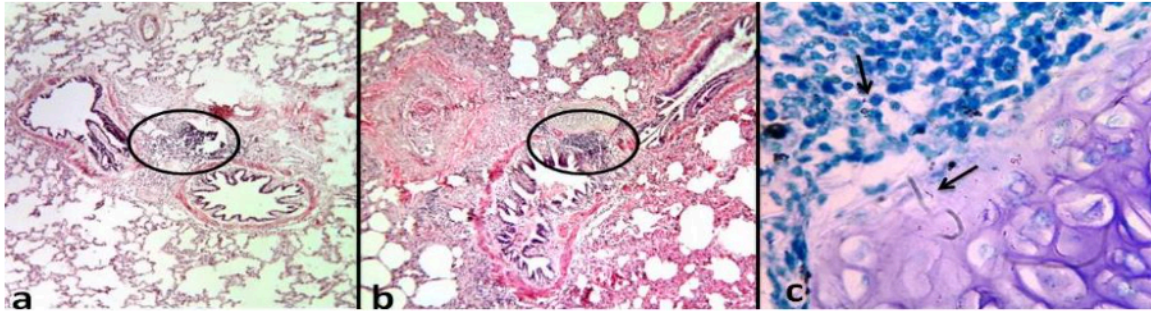


Figure 3: H&E (a and b, 10x) and Acid fast staining (c, 100x) of lung tissue sections. Under microscopic examination granulomas in lungs (a and b, circle) was seen. The epithelioid cells of the granulomas and its surrounding tissues found to contain acid fast bacteria (c, black arrow).

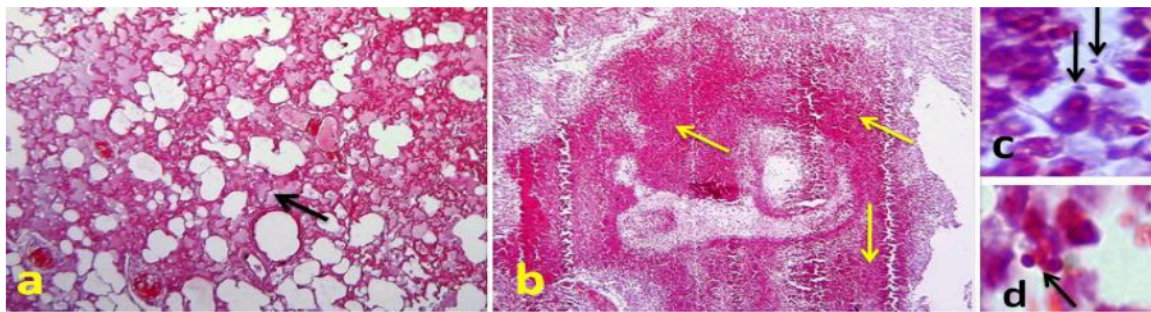


Figure 4. Gram staining of the sections of lungs of a slaughtered bull. There were pink color copious exudates (a, arrow, 10x) in the lungs alveoli. Abscess formation was seen (b, 4x) in multiple foci of the lung tissues containing pink staining purulent materials (yellow arrow). Under high power microscopic field (c, 100x and d, 200x) Gram positive bacteria was seen (arrow).

Tissue sections of liver stained with H&E showed wide spread cirrhosis (Fig. 5a) and formation of granuloma (Fig. 5b). Nahar *et al.* (2011) reported cirrhotic liver with granulomatous reaction in tuberculosis infection in cattle. There was abscesses in liver, the liquefied necrotic mass disappeared at time of tissue processing and appeared empty at time of examination under microscope. The wall of the abscess stained

intensely pink and consisted of neutrophils and fibrous connective tissues (Fig. 5c). Section of pipe stem liver stained with H & E showed the presence of flukes inside the intra-hepatic bile duct (Fig. 6a). There was hemosiderin pigment in the Kupffer cells of liver. Hyperplasia of bile duct and duct epithelium was also seen in the affected liver (Fig. 6b, c).

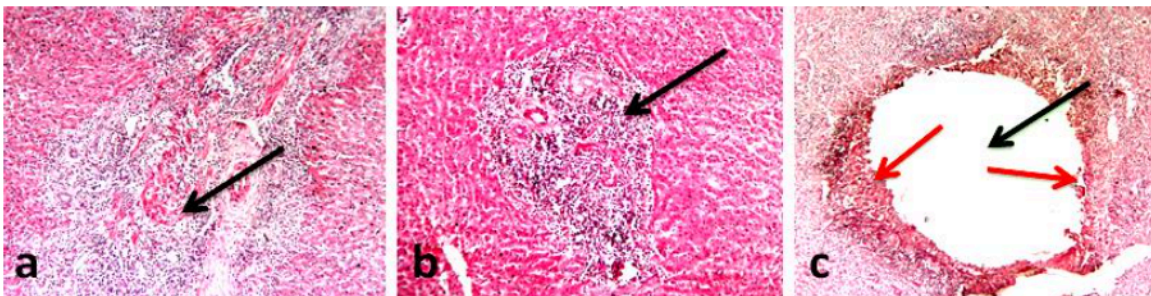


Figure 5. H & E staining of the sections of liver showed wide spread cirrhosis (a, arrow, 10x) and formation of granuloma (b, arrow, 20x). In a liver, there was abscess formation (c, 4x), the necrotic mass disappeared at time of staining and appeared empty (black arrow). The wall of the abscess stained intense pink (c, red arrow) consisting of neutrophils and fibrous connective tissues.

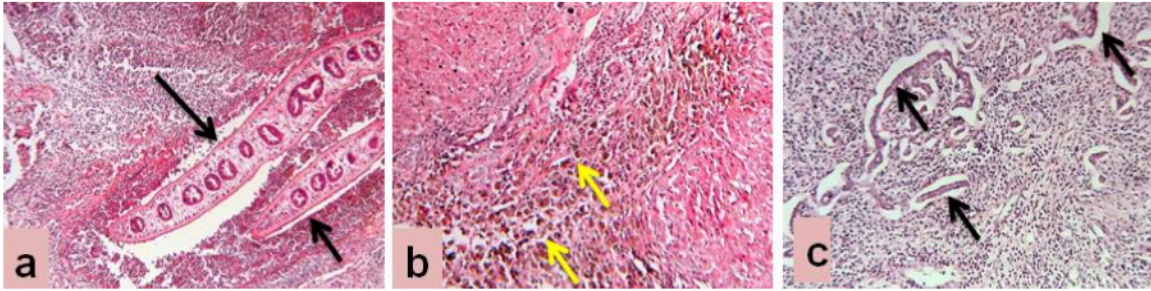


Figure 6. Sections of liver stained with H & E. Sections of liver fluke (a, arrow, 4x) in intrahepatic bile ducts, hemosiderin pigment in Kupffer cells (b, arrow) and hyperplasia of bile duct and duct epithelium (c, arrow, 10x) was seen.

While, sections of spleen stained with H & E and Gram stains showed distended trabeculae with lymphocytic depletion indicating atrophy of the spleen in three cattle (Fig. 7a, b, c). Dey *et al.* (2013) reported in histopathological study that atrophy of spleen in cow and aborted fetus is related to *Brucella* infection. To detect bacteria in

tissues, special staining is required to carry out. Gram staining of kidney section did not reveal any bacteria either in the tubular lumen or in the interstitium. The evidence of the presence of *Brucella* or *Leptospira* could not be confirmed by using Gram staining method.

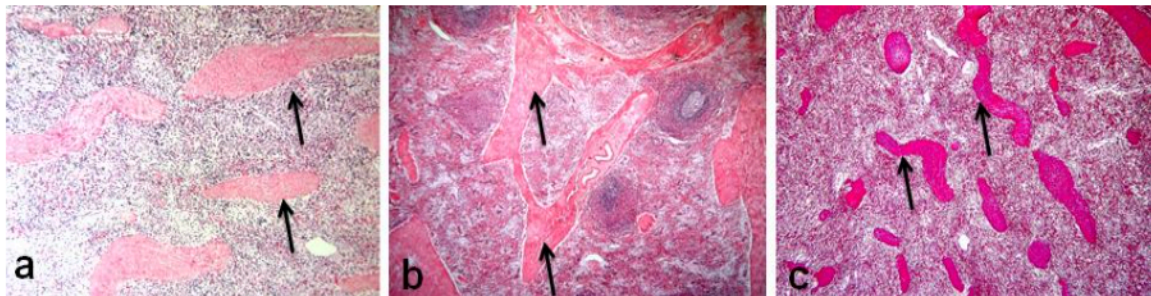


Figure 7. Sections of spleen stained with H & E (a, 10x and b, 4x) and Gram staining (c, 4x). In three cattle the trabeculae (arrow) of the spleen appeared thicker with lymphocytic depletion indicating atrophy of the spleen.

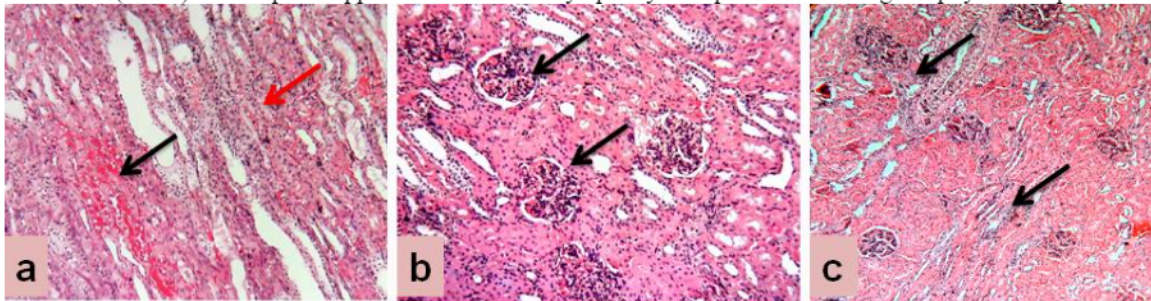


Figure 8. Sections of kidneys stained with H & E (10x). In general nephritis (a, red arrow) and glomerulonephritis (b and c, arrows) was seen in all of the kidneys. Hemorrhages in kidney parenchyma (a, black arrow) was also seen.

Polymerase Chain Reaction (PCR)

To detect specific etiology of *Mycobacterium*, *Brucella*, *Leptospira* and *Toxoplasma* in tissues, DNA was extracted from lungs, liver, kidney and spleen. Specific primers were used in PCR to

identify these organisms. Out of 20 cattle investigated, one cattle showed positive amplification (346 bp) for *Mycobacterium spp* (Fig. 9). The primer was designed targeting the H37Rv strain of *M. tuberculosis* and therefore,

the organism detected reasonably was *M. tuberculosis*. This study provides evidence that PCR is a sensitive screening assay for the detection of *M. tuberculosis* with DNA obtained

from lungs and can generally be used to diagnose bovine tuberculosis under field condition (Sreedevi and Krishnappa, 2003, Taylor *et al.*, 2007).

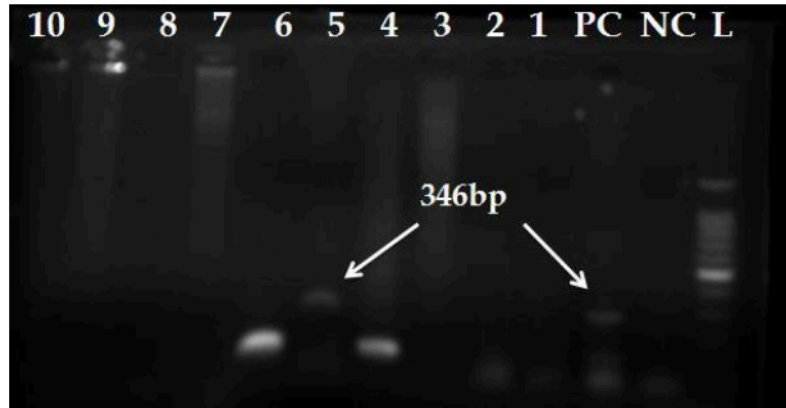


Figure 9. PCR detection of specific cause of Tuberculosis with the DNA extracted from the lungs of cattle. The lane L is for 100 bp ladder, NC is for negative control, PC is for positive control and lane 1 to 10 are for test samples. PCR specific to *M. tuberculosis* was detected in a case (346 bp, lane 5).

However, existence of *M. bovis* or other species of *Mycobacterium* was not validated. Sequencing of the PCR amplicons and sequence analysis may enable us to confirm the species specificity of the organism targeted. Four sets of primers were designed to detect *Mycobacterium*, *Brucella*, *Leptospira* and *Toxoplasma*. However, none of the tested samples showed specific amplification in PCR for brucellosis (alkB/621), leptospirosis (HP/323) and toxoplasmosis (B1/512). It needs to test large number of samples in PCR settings and other molecular technique like ELISA to know the presence of *Mycobacterium*, *Brucella*, *Leptospira* and *Toxoplasma* organisms in various tissues of slaughtered cattle to unveil their existence of those diseases in slaughtered cattle.

Conclusions

Fascioliasis, hydatidosis and tuberculosis like zoonotically important diseases are prevalent in slaughtered cattle of Bangladesh. PCR was found as a sensitive and specific screening test to diagnose tuberculosis. Screening of large number of samples is necessary to detect the presence of important zoonotic diseases and their level of

infectivity in slaughtered cattle in order to design future preventive or control strategies.

Conflict of Interests

The authors declared no conflict of interests.

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