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Research Article
Standardize Polymerase Chain Reaction (PCR) Technique for the Detection of Pathogenic Serovars of *Mycobacterium a. avium* Infection in Layer Chicken

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Abstract

Objectives: Avian tuberculosis (ATB) is caused by *Mycobacterium a. avium*, potentially zoonotic and requires adapting molecular techniques to detect pathogenic serovars and prevent zoonosis. **Methodology:** Layer chicken (N = 2000) of organized poultry farms of Mymensingh district showed clinical signs of progressive emaciation and reduced weight gain constituted the study materials. This study used necropsy, histopathology and Ziehl Neelsen staining to identify specific pathology of ATB in chicken. This study adapted a Polymerase Chain Reaction (PCR) technique to detect ATB in layer chicken due to highly pathogenic variant (serovars 1, 2 and 3) of *Mycobacterium a. avium*. **Results:** Investigation of sick birds at necropsy showed granulomas in liver, spleen and intestine and suspected as a case of ATB. Using histopathology, multi-focal accumulation of macrophages, epitheloid cell and lymphocytes were seen in liver, spleen, kidney, heart and intestine. Acid fast bacterium was detected in tissue sections of spleen, liver and intestine using Ziehl Neelsen staining but unable to differentiate infectivity due to pathogenic, low pathogenic and saprophytic variants of *Mycobacterium*. Visceral organs were, therefore, collected for PCR detection of specific cause of ATB. A specific PCR protocol was adapted targetting 16S rRNA gene (192 bp) and successfully detected pathogenic variant of ATB (*M. a. avium*) in clinically infected and carrier chickens. **Conclusion:** The PCR technique showed the potentiality to diagnosis pathogenic variant of ATB in a few hours with high degree of sensitivity and specificity. Pathogenic variant (serovars 1, 2 and 3) of ATB is highly contagious and potentially zoonotic. The PCR technique can be used to screen elderly layer chickens, diagnose ATV at early onset and dispose the infected flock to prevent future zoonosis.

Key words: Avian tuberculosis, necropsy, histopathology, Ziehl Neelsen staining, PCR, pathogenic variant

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Commercial poultry farming in Bangladesh now-a-day has flourished at wider scale to meet the increase demand of protein by the huge sum of people[1]. Village poultry are usually regarded as a "Walking Bank" or "Bank Coin" for the poor families. Poultry played a pivotal role in the subsistence economy and contributed more than 1.6% in GDP[2] in Bangladesh. However, the farmers lack knowledge on different aspects of diseases and their management. Regular outbreak of infectious and non-infectious diseases of poultry in Bangladesh hindering profitable agriculture. Report onto the outbreak of avian tuberculosis (ATB) in poultry industry of Bangladesh is rare.

Avian tuberculosis (ATB) is an important zoonotic and contagious disease of most warm-blooded animals and birds and caused by an intracellular acid-fast organism *Mycobacterium* sp. Avian TB, a list B disease according to World Organization for Animal Health, caused by *M. avium* subsp., *avium* (*M. a. avium* serovars 1, 2 and 3) and *M. intracellulare* (*M. avium* serovars 4 and 20), predominantly affects poultry and pet or captive birds[3]. The *M. a. avium* is a highly pathogenic bacterium, whereas, *M. intracellulare* has little virulence for chickenss[4,5]. Clinical manifestations in birds due to *M. a. avium* include emaciation, depression and diarrhea along with marked atrophy of breast muscle. Unlike typical caseous necrosis in lungs of animals and man, such lesions in lungs are scanty in ATB. Tubercular nodules can be seen in liver, spleen, intestine and bone marrow. Granulomatous lesion without calcification is a prominent feature. The disease is a rarity in organized poultry sector due to improved farm practices, but common in zoo aviaries. It needs to know the occurrence of ATB due to highly pathogenic or little virulence bacterium to design future spread and possible zoonosis. Molecular techniques like Polymerase Chain Reaction (PCR) combined with restriction fragment length polymorphism and gene probes aid in rapid identification and characterization of mycobacteria subspecies and overcome disadvantages of conventional methods which are slow, laborious and may at times fail to produce precise results. The *M. a. avium* with genotype IS901+ and IS1245+ causes infections in animals and human. The bacterium causes sensitivity in cattle to the tuberculin test[6].

Avian TB caused by *M. a. avium* belonging to serovars 1, 2 and 3 (genotype IS901+ and IS1245+). Other species, such as *M. genavense*, *M. intracellulare*, *M. scrofulaceum*, *M. fortuitum*, *M. tuberculosis* and *M. bovis* can also causes TB in birds, but the incidences are rare in clinical form[7]. The *M. a. avium* causes ATB probably in all avian species including waterfowl, galliformes, columbiformes, passerines, psittacines, raptors and ratites[7,8]. The disease is distributed worldwide but most frequently seen in the North Temperate Zone[9] of the globe.

Susceptibility to disease varies from species to species. ATB in bird species broadly classified into four groups (a) Highly susceptible: Domestic fowl, sparrows, pheasants and partridges, (b) Less susceptible: Guinea fowl and domestic turkeys, (c) Moderately resistant: Domestic goose and duck and (d) Highly resistant: The domestic pigeon[10]. In avian species, stress factors appear to enhance disease processes and is particularly worthy in case of birds living in captivity[11]. Infected birds and contaminated water and soil are the main source of infection as the mycobacteria can survive for several months in the environment[7]. The disease is more prevalent in places with high population density and poor sanitation and hygienic conditions. The practices of allowing birds to roam freely and keeping the breeders for several years are highly conducive to the spread of tuberculosis[12]. A flock once infected with ATB, they showed unthriftiness, decreased egg production and increased mortality, which culminates total loss due to stumping out of infected farm.

Mycobacterium avium complex (MAC), comprising *M. avium* subsp., *avium* (*M. a. avium*), *M. avium* subsp., *paratuberculosis* (Para TB), *M. avium* subsp., *silvaticum* and *M. intracellulare* may also infect different animal species like swine, cattle, deer, sheep, goat, horses, cats, dogs and exotic species besides causing infection in immunocompromized human[13]. The *M. genavense* has also been reported in dogs and immunocompromised cats. The *M. intracellulare* is a closely related pathogen of birds with a lower prevalence[14]. Until now, ATB due to *M. a. avium* is considered as the most devastating pathogen in birds[15,16]. Clinical sign and gross pathological examination are usually considered as an early tool to detect ATB but the confirmatory diagnosis requires molecular technique like enzyme linked immunosorbent assay[17] and Polymerase Chain Reaction (PCR)[18,19]. Due to the similarities in morphology, genomic characteristics and pathology of these organisms it needs to design or adapt technology to specifically detect variant of organisms involved. For successful execution of these considerations this study adapted a PCR protocol to detect pathogenic variant of ATB in layer chickens and compared its efficacy with other available traditional technologies.

MATERIALS AND METHODS

Clinical examination and necropsy: The study was conducted in the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU),

Mymensingh. Layer chickens (N = 2000) in various farms of Mymensingh district, Bangladesh with a history of progressive weight loss, suspected as a case ATB and constituted the study materials. These cases were investigated extensively to identify the pathogen and specific cause of illness. A total of five clinically infected layer chickens (about 45 weeks of age) and five apparently healthy layer chickens from these farms was investigated. The clinical examination was carried out in farm and necropsy was carried out with extreme care to avoid zoonotic spread. Gross tissue changes observed were recorded by systemic dissection. The tissue samples were collected in 10% neutral buffered formalin for histopathological investigation. Portion of liver, spleen and intestine was snap frozen for the extraction of genomic DNA and PCR detection of the variant of *Mycobacterium*.

Histopathological examination of lesions: Formalin-fixed lungs, liver, spleen, muscle, kidney and intestine from the suspected layer chickens were processed for paraffin embedding, sectioning and staining with haematoxylin and eosin (H and E) and Ziehl Neelsen staining[20]. The stained tissues on to the slides were examined at low (10x) and high (100x) power microscopic fields, images were captured and analyzed.

PCR detection of specific cause of ATB: The genomic DNA from liver, spleen and intestine of infected and carrier birds were extracted using traditional methods. Briefly individual tissue (2 g) was crushed in liquid nitrogen, dissolved in 600 µL of cell lysis buffer, transferred in an eppendorf tube and vortexes to wet the tissues. The solution containing tissue lysate was incubated at 56°C for 2 h. The solution in the tube was centrifuged at 1000 g for 5 min and supernatant was collected. Equal volume (400 µL) of phenol chloroform isoamyle alcohol (PCI) was added, vortexed and spin down at 10000×g for 2 min. Supernatant (top layer, 300 µL) was collected in a fresh tube, added 1/10th volume of 5 mol NaCl and 2.5x ice cold ethanol. The mixture was incubated on ice for 30 min and extracted DNA using standard procedure[21]. The DNA samples were evaluated both quantitatively and qualitatively using spectrophotometer (A^{260}-A^{280}) and agarose gel electrophoresis, respectively. The concentration of extracted DNA obatined were between 200-300 ng μL^{-1} of solution.

A uniplex PCR (192 bp) was adapted in a 25 µL reaction volume to detect fragment of 16S rRNA gene of *M. avium*. Forward (AGAGTTTGATCCTGGCTCAG) and reverse primers (ACCAGAAGACATGCGTCTTG), 20 pmol of each were used[18] per reaction in a programmable thermocycler using PCR Kit (Promega, USA). The thermal profile consisted of an initial denaturation for 4 min at 95°C followed by 40 cycles of DNA amplification reaction in a Master Cycler (Master Cycler Gradient, Eppendorf, Germany). The condition of PCR amplifications were denaturation for 60 sec at 95°C, annealing for 90 sec at 56°C and extension for 60 sec at 72°C followed by a final extension for 10 min at 72°C. The PCR reactions were held at 4°C and the reaction was terminated by adding 3 µL of 50 mM EDTA. The PCR products were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide and examined under UV light using an image documentation system (Cell Biosciences, Alphalmager HP, USA).

RESULTS AND DISCUSSION

Several mycobacterial species are involved with the tuberculous lesions in birds. Avian TB is mostly caused by highly pathogenic *M. a. avium* (serovars 1, 2 and 3), low pathogenic *M. a. avium* (serovars 4-20 known as *M. intracellulare*) and *M. genavense*. Highly sensitive PCR technique is required to selectively detect infectivity in birds due to a specific serovar. The incidence of ATB in layer chickens is not a regular finding and there are limited literature describing the incidence of ATB in chickens in Bangladesh. The incidence of ATB in pet birds is higher and farm chickens may catch infection from the pet birds[22]. Some of the reasons of the incidence of the infection in pet birds are age of the host, population density and the ability of organism to survive environmental inclemency[7]. The primary lesions of ATB in birds mostly seen in the intestinal tract. Intestinal lesions take the form of deep ulcers filled with caseous material containing many *Mycobacterium* and these are discharged into the lumen and appear in the faeces. Similar findings are also described earlier[23] and reported that fecal contamination of water, soil, or feed thus enabling transmition of infection[24]. However, the source of infection in the layer farms was not identified but free living rodents, crows or other birds may play role in disease transmission. According to OIE[25] the infectivity in pet birds or captive chicken can be identified by using tuberculin test or serological tests. If acid-fast bacilli are not seen in tissues but typical tuberculous lesions are present in internal organs of birds, culture of the organism was suggested. However, nucleic-acid-based technologies like PCR is the most sensitive and recommended technology to detect and differentiate causal agents of ATB with the DNA extracted directly from tissue samples. In this study infectivity of layer chickens due to ATB was made by using traditional techniques like clinical

signs, necropsy, routine H and E staining and Ziehl-Neelsen staining of tissue sections. A uniplex PCR was adapted for specific detection of pathogenic variant of ATB and compare the efficcacy of traditional techniques with the PCR in terms of sensitivity and specificity to detect pathogenic variant of ATB in layer chicken.

History, clinical sings and gross pathology: In most cases, infected birds showed little sign of illness, but they may eventually become lethargic and emaciated. Many affected birds showed diarrhoea, comb and wattles may regress and become pale. Under intensive husbandry conditions, sudden death may occur, often associated with severe lesions in the liver; such lesions are easily observed at necropsy. Similar findings were also described by the World Assembly of Delegates[25] of the OIE in May, 2014 and suggested that detection of ATB in elderly captive, wild or pet birds are needed to diagnose with care. In this study the farms investigated have had about 2000 layer chickens and during on farm investigation, about 45 birds showed signs of illness comprising emmaciation, drop of egg laying, unthriptiness and reduced feed intake. The affected chickens at necropsy appeared cachectic (Fig. 1a), showed thin musculature and prominent kill bone manifested as "Knife edged" (Fig. 1b). Some birds were adapted in sitting position. Jerky hopping gait with unilateral or bilateral lameness was observed in 10-12% affected chickens. Thoen[5] suggested that the lameness in infected birds was due to tuberculous lesions in the leg bones and joints but such lesions in bone and joint was not seen in this study. About 80-85% birds in the infected farm were apparently healthy except a history of progressive weight loss. The layer birds at their age of 45 weeks stop laying eggs and this was due to loss of body condition. Case fatality was seen in 4-5% cases due to rupture of liver and or spleen and massive congestion in lungs. Previous study[9] also described such lesions in liver and spleen but the case fatality described was a bit lower (2-3%) than the present study. In some case, the birds were died suddenly in good bodily condition and without advanced lesions of tuberculosis. Tell *et al.*[23] reported that the death was due to multiple organ failure and appeared similar with the finding of this study. The body temperature of the affected bird was normal, even in severe cases. There were multiple whitish nodular masses on liver (Fig. 1c, 2a) and enlargement of spleen (Fig. 2b), these are typical lesions of avian TB[26]. Affected part of intestine especially ileum appeared thicker (Fig. 2c), containing whitish nodular lesions and diffuse thickening in various part of ileum. Typical nodular lesions as seen in the

Fig. 1(a-c): Fourty five weeks old layer chicken (a) Obtained from a commercial farm showed progressive weight loss, muscular atrophy and prominent kill bone, (b) Black arrow at necropsy and (c) Liver of the suspected chickens were larger and containing multiple diffuse whitish necrotic patches

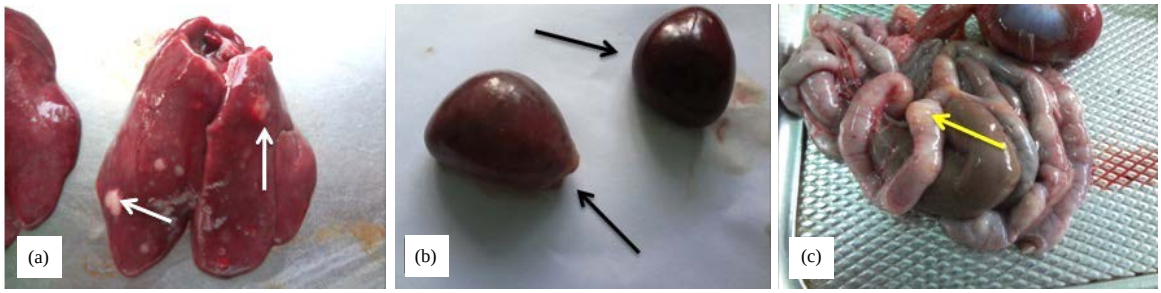

Fig. 2(a-c): Pathological changes observed in the (a) Liver, (b) Spleen and (c) Intestine of the suspected layer chickens. There were numerous whitish nodular lesion embeded in liver (a, white arrow). Spleen of infected chickens appeared larger (b, black arrow) and there was diffuse thickening in various part of ileum (c, yellow arrow)

liver and spleen, leading to enlargement of the organs was due to the formation of new granulomas. Previous studies described that the lungs of ATB infected birds was ordinarily free from granulomatous lesions even in advanced cases[13,23] but massive congestion as seen in the lungs of infected birds was not described in previous study.

The occurance of TB in free living birds[27] including raptors were reported earlier, describing disseminated form where lesions were distributed in multiple organs including digestive tract, liver and spleen. Gross lesions of the affected layer chicken as seen in this study could be a disseminated form of ATB and involving multiple organs like spleen, liver, kidney, heart and intestine. Nodular lesion was not seen in lungs, heart and skeletal muscles. The pneumonic lesions as seen could be due to secondary to other bacterial infection. Pulmonary avian TB is only occasionally reported as in case of TB of water fowl[8]. Based on the lesions, the disease process was described in three phases: Latency, lesion development and period of cachexia. During cachexia, massive granulomas in visceral organs was seen. In the classic form of infection, which is seen in this study the tubercles or granulomas develop in multiple organs; a second form is manifested with lesions in the intestinal tract; a third type of infection often reported as diffuse granulomas, mainly seen in finches, canaries and psittacines. Previous studies[23,25] also described avian TB in three phases but the typical lesions in each phases varies in various cases and state of management. Some birds occasionally showed respiratory signs and sudden death, dyspnoea was less common but granulomatous ocular and skin lesions were prominent[28]. The lungs of infected chickens were congested and consolidated could have microscopic lesion and were studied by means of histopathology.

Histopathological investigation: Sections of lungs, liver, spleen, kidney, heart and ileum stained with H and E staining showed multi-focal accumulation of macrophages, epitheloid cell, lymphocytes and fibrous connective tissue. Accumulation of macrophages and lymphocytes and deposition of fibrin was seen in liver (Fig. 3a), spleen (Fig. 3b) and heart (Fig. 3c). The epithelial lining of affected ileum was necrosed and slaughed off (Fig. 4a). There were accummulation of macrophages and lymphocytes in the mucosa (Fig. 4b, c) of intestine. Characteristics granulomatous reaction containing caseous necrotic center as seen in mammalian TB was seen in the central lesions of ATB. Nodular lesion was not seen in the sections of lungs but containing scatterdly distributed lymphocytes and macrophages. Similar changes in lungs of chickens infected with ATB was reported earlier[29,30]. Congestions and hemorrhages in the lungs of infected birds was seen and was due to infection with *Pasteurella* spp.,

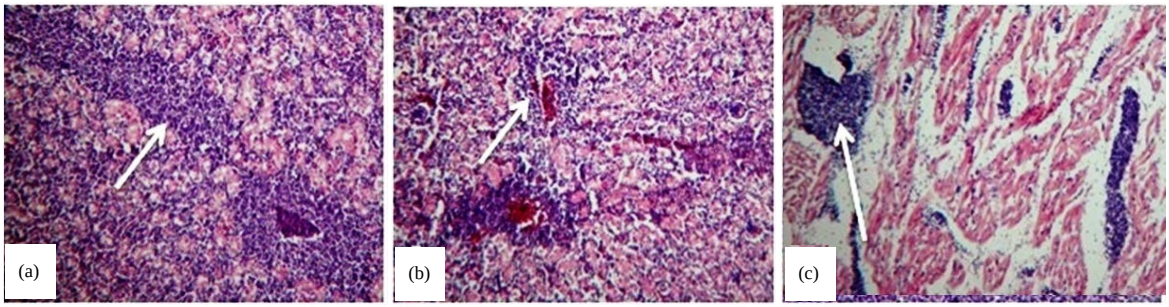

Fig. 3(a-c): Section of (a) Liver, (b) Spleen and (c) Heart stained with hematoxylin and eosin. There were multifocal accummulation of mononuclear cells (white arrow) in these organs indicating chronic granulomatous reaction (10x)

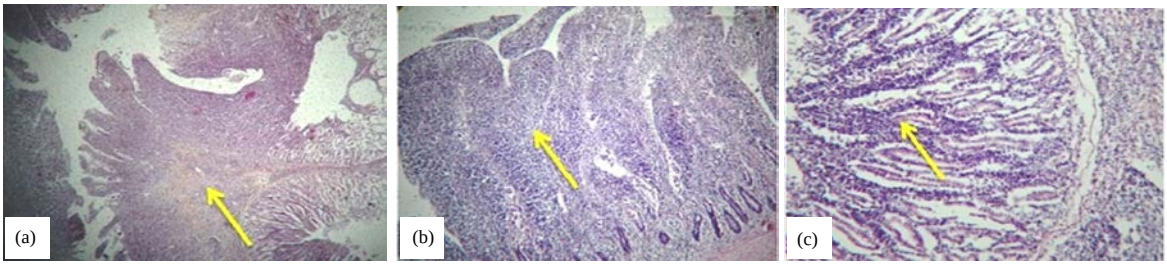

Fig. 4(a-c): Section of ilium of suspected layer chickens stained with hematoxylin and eosin showed diffuse thickening of the mucosa of illium (a) Yellow arrow, 4x, profuse infiltration of mononuclear cells in this intestinal villei, (b) Yellow arrow, 10x and degeneration and necrosis of intestinal mucosa and (c) Yellow arrow, 40x

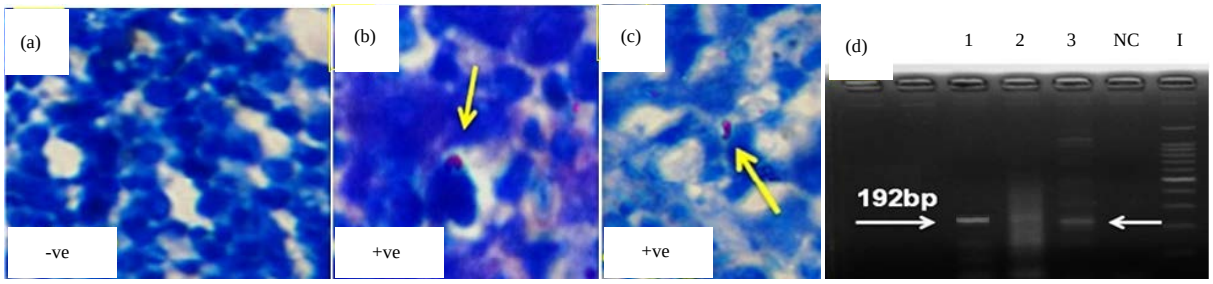

Fig. 5(a-d): Ziehl-Neelsen staining of acid fast bacteria in the section of (a) An infected (-ve) and uninfetced chickens of (b) Liver and (c) Spleen. Light pink color comma or rod shaped acid fast bacteria was seen in the cytoplasm of macrophages (b and c, yellow arrow, 100x) which, however, was absent in the (a) Control section. Using PCR 192 bp ampliocn specific to highly pathogenic serovar of *M. a. avium* was generated with the DNA extracted from the intestine (lane 1), liver (lane 2) and spleen (lane 3)

(common isolated from lungs lesion). Pulmonary form of avian TB due to *M. avium* is not a regular finding in layer birds but occasionally seen in pigeons and water fowl[5].

However, characteristic caseous necrotic center was not seen in all of the organs investigated, this could be due to disseminated form of TB. Disseminated form of ATB involving the digestive tract, liver and spleen of free living birds including raptors was predominant[27]. The H and E staining of tissue section showed disseminated form of TB with granulomas in infected organs; such granulomas can also be seen in a number of diseases like coligranulomas, *Heterakis gallinarum*, aspergillosis etc., hence differential diagnosis is required. In order to detect acid fast bacteria in the lesion and to ensure infectivity due to acid fast bacilli, the Ziehl-Neelsen staining of tissue sections were carried out.

Ziehl-Neelsen staining of the sections of lungs, liver, spleen, kidney and intestine showed pink color acid fast bacteria in the epitheloid cells/macrophages of affected organs. Very low densities (0-1 bacteria per 100x objective) of infiltrating epitheloid cells/macrophages was seen in kidney, moderate densities in liver (1-3 organism per 100x objective, Fig. 5b) and higher densities (2-5 organism per 100x objective) in spleen (Fig. 5c). Acid fast bacteria and granuloma was not seen in the lungs of infected chickens. The sections of liver, spleen, kidney and intestine of apparently healthy chickens although showed sactterdly distributed inflammatory lesions, acid fast bacteria was not seen. The epitheloid cells in the central part of granulomas containing large numbers of pink color bacilli. Ziehl-Neelsen staining[9,26] is a special staining technique has been used to detect acid fast bacilli in tissues but cannot detect acid fast bacteria in subclinical form. Moreover, Ziehl-Neelsen staining of tissue section although detect acid fast bacterium in epitheloid cells but unable to differentiate infectivity due to variant serovars of ATB. The highly or low pathogenic variant of ATB involved was, therefore, detected using PCR.

PCR detection of ATB: Specific and reliable tests for speciation are the use of nucleic acid based technology. Commercial nucleic acid hybridisation probes have become a 'Gold standard' for distinction between *M. a. avium* and *M. intracellulare*[31]. The *M. genavense* can also be distinguished using nucleic acid hybridization probes. A concern is that the nucleic acid hybridisation probes could have led cross-reactivity among serovars of *M. a. avium*, which may have serious consequences[32]. Various in-house molecular methods have been reported to design or adapted for the identification of specific causes of ATB. The PCR method targeting fragment of 16S rRNA gene for differentiating *M. avium* from *M. intracellulare* and *M. tuberculosis* complex has got some advantages. The PCR technique adapted in this study was initially designed to amplify fragment of 16S rRNA gene (192 bp) of a highly pathogenic variant of *M. a. avium*[8,18]. Results of PCR showed amplification of 192 bp fragment with the DNA extracted from intestine, liver and spleen (Fig. 5d) of clinically infected layer chickens. Such amplification was also seen with the DNA directly collected from asymptomatic birds. The clinically infected and susceptible layer chickens investigated in this study was infected with highly pathogenic variant of ATB. The ATB in layer industry due to pathogenic variant of *M. a. avium* causes higher rate of morbidity and lower rate of mortality. Such higher rate of morbidity and mortality in infected birds due to *M. a. avium* also described by Tell *et al.*[23] and OIE[25] and appeared similar with the findings of this study.

Avian TB and zoonosis: Among domestic mammals, pigs are most susceptible to ATB but clinical manifestations usually not seen. Avian TB in pigs is suspected while tuberculous lesions are seen in the lymph nodes (head and mesenteric) on meat inspection. Tuberculous lesions in pigs due to *Mycobacterium a. avium* usually seen at advanced stage of the disease and accounted for up to 35% of the mycobacteria isolated from such tuberculous lesions in Czech Republic[33]. Cattle although resistant to the causative agent of avian TB, the tuberculous lesions due to *Mycobacterium a. avium* can be detected in mesenteric lymph nodes and liver on meat inspection. The isolation rate of *Mycobacterium a. avium* in cattle at slaughter over 2 years of age ranged[34] from 34.4-13.0%. However, all members of *M. avium* complex and *M. genavense* are capable of infecting humans that is refractory to treatment, especially in immunocompromised individuals[23,35]. Members of *Mycobacterium avium* complex are classed in risk group 2 for human infection and should be handled with appropriate measures as described[25]. The ATB was a significant threat of infectivity to poultry, human, cattle, pigs, other captive or free living birds with condemnation of carcasses during 2000-2010[3,8,9,14,27,31]. During the last few years, there were few literatures describing the incidence of ATB in organized poultry farm as detected by necropsy and PCR[25,36,37]. OIE Terrestrial Manual 2014 addressed the etiology, pathology, diagnosis and zoonotic spread of ATB in the World Assembly of Delegates[25] under the OIE in May, 2014. The incidence of ATB in poultry farms attract less attention by the researcher in developed countries due to proper biosecurity measures in farm operation. Due to use of unlawful farm chemicals, feed additives, feed preservatives, anticoccidials, toxin binders, growth promoters, antibiotics etc., in the poultry feed in developing country; the ATB may have re-emerged and transmitted to other animals and birds. Detection of human TB due to *M. a. avium* serovars 1, 2 and 3 has got little attention in most of the world including Bangladesh. It requires designing multiplex PCR to detect members of mycobacterium avium tuberculosis complex and effectively discriminating closely related mycobacterium species. The suspected poultry, farm workers, pet birds, pet animals, etc., found in suspected/infected premises requires veterinary and public health intervention to diagnose ATB at early onset and designing future diagnostic, treatment and preventive strategies accordingly.

CONCLUSION

- The principal lesions of ATB as seen at necropsy were dry and emaciated muscle (knife edged), greyish-white to greyish-yellow nodules in intestine, liver and spleen. A disseminated form of ATB in layer chickens was seen in this study
- The definitive diagnosis of ATB relies onto the use of PCR protocol, most sensitive, rapid and accurate tool to identify pathogenic serovars of *M. a. avium* within short period of time
- The PCR protocol successfully detected both the clinical and subclinical form of ATB especially the pathogenic serovars within shortest period of time
- Tuberculosis in dairy cattle and vulture due to *M. a. avium* was also detected in our laboratory. The suspected flocks require to examine for the presence of ATB by using PCR and the infected flock should be culled with extreme care. Human being working in the farms requires to sit for ATB testing by regular interval to prevent future casualty
- Once ATB is detected in a farm, the farm premises require disinfecting to prevent future dissemination and human casualty

SIGNIFICANCE STATEMENT

- Commercial poultry (about 50-55% of whole chicken population) in Bangladesh has grown a lot to meet the demand of meat and eggs
- The level of biosecurity measure of commercial and native poultry in Bangladesh is very low. The infectious diseases confronting a major hurdle of profitable poultry production in Bangladesh
- Recently incidence of avian tuberculosis (ATB) in organized poultry farms is increasing but the issue left unaddressed due to lack of appropriate technology
- This study adapted nucleic acid based (PCR) technology to detect Pathogenic variant of ATB (*M. a. avium*)
- Layers in farming system were infected with ATB and hindering income generation due to culling of infected chickens and disinfectant of farm premises
- ATB is detected in layers, also identified in dairy cattle and vulture and posed threat to other susceptible animals (pigs, cattle and human)

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