Histopathological study of avian tuberculosis in naturally infected domestic pigeons with Mycobacterium avium subsp. avium

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Abstract

The aim of this study was to investigate the histopathology of avian tuberculosis in naturally infected domestic pigeons (Columba livia var. domestica) with Mycobacterium avium subsp. avium. Avian tuberculosis is one of the most important diseases that affect all species of birds, and is most often caused by Mycobacterium avium and Mycobacterium genavense. 80 out of more than 600 pigeons were selected based on their clinical signs and poor health conditions and under standard conditions were euthanized, necropsied, followed by bacterial culture on specific media for Mycobacterium avium subsp. avium. Fifty Mycobacterium avium subsp. Avium were isolated from pigeons. All acid-fast bacilli isolates were tested by the PCR assays targeting the 16S rRNA, IS1245 and IS901 genes. After definitive identification of Mycobacterium avium subsp. avium by culturing and PCR assay, 45 fixed samples including liver, gizzard, proventriculus, intestines, kidneys and lungs from positive pigeons were subjected for histopathology studies. Tissues sections were prepared as usual and stained by haematoxylin and eosin, Ziehl-Neelsen and Congo red. Based on gross findings, liver and intestines were the most affected organs. Histologically, caseative uncalcified granulomatous inflammation was noticed in the affected organs. Also histopathology examinations showed that most of the granulomatous lesions in the lungs were in microscopic size and it seems that lungs were affected more than it was expected. In Ziehl-Neelsen’s staining, a large number of acid-fast bacilli were observed within multinucleated giant cells and in necrotic areas. Also in Congo red staining, deposition of amyloid in liver and kidneys sections were observed. In conclusion, histopathology findings were typical of avian tuberculosis, including acid-fast bacilli and uncalcified caseous necrosis centers that were surrounded by multinucleated giant cells, macrophages and lymphocytes.

Keywords: Pigeon, Mycobacterium avium subsp. avium, amyloid, granulomatous lesions, acid-fast bacilli

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Introduction

Avian tuberculosis is one of the most important diseases that affect most orders of birds (Van darheyden, 1997 and Tell et al., 2001). Several mycobacterial species can be involved in the etiology of avian tuberculosis. The disease is most often caused by *Mycobacterium avium* belonging to serotypes 1, 2 and 3 (genotype IS901+ and IS1245+) and *M. genavense* (Dvorska et al., 2007 and Fulton et al., 2008). All species of birds can be infected with *Mycobacterium avium*. Domesticated fowl or captive wild birds are affected more frequently than those living in a wild state (Fulton et al., 2008). Symptoms of mycobacteriosis in birds include chronic illness characterized by weight loss, diarrhea, dyspnea, lameness and poor feathering even though a significant number of birds die acutely without recognized symptoms (Van darheyden, 1997). The most common route of infection for susceptible birds is via the alimentary tract; however, pulmonary avian tuberculosis and egg transmission have also been described (Thorel et al., 1997 and Dvorska et al., 2007). Lesions are seen most frequently in liver, spleen, intestines and bone marrow and less infrequently in the other organs. Gross lesions consist of irregular grayish-yellow or grayish-white masses, which are firm but easily incised (Thorel et al., 1997 and Cowper et al., 2007). Microscopically, lesions of avian tuberculosis consist of a central necrotic core surrounded by epithelial macrophages, lymphocytes, multinucleated giant cells and a fibrous capsule. Calcification is rarely seen in birds. Amyloid deposition occurs mainly in the liver, but is also seen in the spleen, blood vessels and parenchyma of many organs (Tell et al., 2001 and Fulton et al., 2008). Acid-fast staining of granulomatous tissues typically reveals large numbers of acid-fast bacilli in contrast to other *Mycobacterium* spp, such as *M. bovis* and *M. tuberculosis*, in which organisms are rare within tubercles (Tell et al., 2001). Because of the importance of avian tuberculosis in avian diseases and the risk of the zoonotic disease, this motivated our interest to investigate the histopathology of avian tuberculosis in naturally infected domestic pigeons (*Columba livia var. domestica*) with *Mycobacterium avium* subsp. *avium*.

Materials and methods

Eighty suspected pigeons (*Columba livia var. domestica*) to avian tuberculosis, out of more than 600 pigeons from more than 10 lofts, were selected based on their clinical signs including swollen joints, lameness, emaciation, tubercle formation under the skin, granulomas in the conjunctival sac and poor health condition were collected. The birds were euthanized and subjected to necropsy examinations. Gross lesions observed in the internal organs were noted on the working sheets and immediately tissues of each bird were aseptically collected in 50 ml screw cap containers and sent to the tuberculin department of the reference laboratory in dry ice chambers for definitive identification. Subsequently, tissue samples of 58 pigeons which they had macroscopic necropsy lesions were taken and fixed in 10% neutral buffered formalin for histopathology examinations.

Mycobacterial isolation

After thawing the tissue samples in the tuberculosis reference laboratory, approximately 4 grams of tissues of each bird were pooled and ground in a pestle and mortar containing sand using sterile materials and equipments. The homogenized mixtures were decontaminated according to the NALC-NAOH method (Salfinger et al., 1987). The inoculums were cultured on Lowenstein Jensen and Herrold egg media. The inoculated slopes were incubated at 41°C for 8 to 12 weeks. Genomic DNA of all isolates from each infected pigeons was extracted according to the van soolingen method (Van Soolingen et
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All acid fast isolates (Kantor et al., 1998) were tested by the PCR assays targeting the 16S rRNA gene for identification of mycobacterium members, IS1245 for mycobacterium avium complex and finally IS901 for identification of Mycobacterium avium \textit{subsp. avium} (Kunze et al., 1991 and Guerrero et al., 1995 and Ikonopoulos et al., 2009). Analyses of PCR products were conducted on ethidium bromide-stained 2% agarose gels in a submerged electrophoresis system.

**Histopathological examinations**

As stated earlier, the samples obtained from the necropsies were fixed in 10% neutral buffered formalin. After definitive identification, 45 fixed samples including 20 liver, and 5 samples each of gizzard, proventriculus, intestines, kidneys and lungs from positive pigeons were selected and processed routinely, embedded in paraffin and sectioned on a manual microtome (Lieca, RM2235, Germany) at a thickness of 4 μm. Then they were stained with haematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN) for the detection of acid-fast bacilli in tissues (Luna, 1968). Also Congo red staining was employed to investigate the presence of amyloid in some of the liver and kidney sections (Luna, 1968).

**Results**

Culturing, ZN staining and molecular identification confirmed that the pigeons were infected with \textit{Mycobacterium avium \textit{subsp. avium}} (Fig. 1 and 2). In necropsy examinations firm grayish-yellow or grayish-white and raised nodules were found especially on liver and intestines (Fig. 3 and 4). Liver and intestines were the most frequently affected organs, and Lungs were the least affected organs while no macroscopic lesion was found in the gonads, kidneys and CNS. In necropsy and histopathology examination of 45 fixed samples, gross and microscopic granulomatous lesions were seen in all livers and intestines and in 2 gizzards and proventriculus, but gross and microscopic granulomatous lesions were seen in one and three lungs, respectively (Table 1). Granulomatous lesions observed in the liver, lungs, gizzard, proventriculus and intestines were characterized by uncalcified caseous necrosis, which were surrounded by numerous multinucleated giant cells (they mainly consisted of foreign body giant cells), macrophages and thick layer of lymphocytes (Figure 5). Also granulomatous lesions observed in the gizzard, proventriculus and intestines were in the serosal layer (Figure 6). In Ziehl-Neelsen’s staining, a large number of acid-fast bacilli were observed within multinucleated giant cells and in necrotic areas (Fig. 7). In liver sections amyloidosis in the wall of some sinuoids and bile ducts (Fig. 8) together with fatty change, cell swelling and cellular atrophy were seen. In kidney sections no granulomatous lesions were observed, but amyloidosis in the wall of uriniferous tubules was seen (Fig. 9).

<p>| Table 1. Comparison of observed lesions in necropsy and histopathological examinations in the organs of infected pigeons with \textit{Mycobacterium avium \textit{subsp. avium}}. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
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<td>Histopathology lesions</td>
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</table>
Figure 1. The example of PCR amplification product. The 427 bp specific fragment from IS1245. Lane M, DNA size marker (100 base pair ladder). Lane 1 and 2, negative controls (distilled water). Lane 3, negative species control (Mycobacterium bovis AN5 strain, ATCC number 35726). Lane 4, positive control (Mycobacterium avium subsp. avium D4 strain, ATCC number 35713). Lane 5 to 9 samples tested for Mycobacterium avium subsp. avium.

Figure 2. The example of PCR amplification product. The 1108 bp specific fragment from IS901. Lane M, DNA size marker (100 base pair ladder). Lane 1 and 2, negative controls (distilled water). Lane 3, negative species control (Mycobacterium bovis AN5 strain, ATCC number 35726). Lane 4, positive control (Mycobacterium avium subsp. avium D4 strain, ATCC number 35713). Lane 5 to 10 samples tested for Mycobacterium avium subsp. avium.
Figure 3. Multifocal granulomatous hepatitis in affected pigeon.

Figure 4. Nodular granulomatous lesions in the intestines of affected pigeon.

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Figure 5. Liver; pigeon. Granuloma with central caseonecrosis (star) ringed by multinucleated giant cells (arrow) and thick layer of lymphocytes (elbow arrow). H&E, ×100

Figure 6. Proventriculus; pigeon. Granuloma in serosal layer of proventriculus, with central caseonecrosis (star) ringed by multinucleated giant cells (arrow). H&E, ×40
Figure 7. Liver; pigeon. Numerous acid-fast bacilli were discovered within in necrotic areas (arrow). Ziehl-Neelsen, ×1000

Figure 8. Liver; pigeon. Amyloid deposition in the wall of bile ducts (arrow). Congo red, ×400
Discussion

A few histopathology studies about avian tuberculosis are presented in the literature (Thorel et al., 1997 and Cowper et al., 2007 and Skoric et al., 2010). Apparently, such studies have never been documented so far in Iran. In present study, all of the isolates carried IS901 insertion sequence, a pathogenicity determinant, and also IS1245 locus. Such isolates that belong to serotypes 1, 2 and 3 of Mycobacterium avium are considered as the most pathogenic strains of Mycobacterium avium in birds (Tell et al., 2001 and Dvorska et al., 2003). In the necropsy examinations, form and color of nodules were consistent with other records of avian tuberculosis lesions (Thorel et al., 1997 and Tell et al., 2003). In all the avian species, the infection is acquired by ingestion; however, an occasional occurrence of aerogenic pulmonary infection has also been described (Shitaye et al., 2010) which correlated with our findings because lung lesions were observed only in one pigeon while liver and intestines were the most affected organs. Only in lungs of one pigeon gross granulomatous lesions were seen, but in histopathology examinations, microscopic lesions were seen in 3 out of 5 lungs. It seems that lungs were affected more than it was expected and most of the granulomatous lesions in the lungs were in microscopic size. These lungs seem to be affected due to secondary hematogenous spread of infection because in other organs of these pigeons granulomatous lesions were seen (Van darheyden, 1997 and Van Soolingen et al., 1997).

In histopathological examinations of granulomatous lesions, caseous necrosis centers which were encircled by numerous multinucleated giant cells, macrophages and thick layer of lymphocyte were observed (Fig. 5) and indicated a complete immune response. In humans, the ability to form multinucleated giant cells is considered one indicator of an effective immune response to tuberculosis (Byrd, 1998 and Smith et al., 2000). Multinucleated giant cells may limit the growth as well as the cell-to-cell spread of Mycobacterium tuberculosis (Byrd, 1998 and North et al., 2004 and Dannemberg, 2006).
Histopathology study of avian tuberculosis in humans with human immunodeficiency virus/acquired immune deficiency syndrome and tuberculosis, poorly developed granulomatous do not have multinucleated giant cells (Smith et al., 2000).

Amyloidosis is a pathological condition that is characterized by the deposition of insoluble fibrillar proteins in various tissues and organs of the body, following prolonged inflammation or infection (Cotran et al., 1999). Amyloid deposits have been reported previously in birds with chronic inflammatory diseases such as Mycobacteriosis and Aspergillosis (Montali et al., 1976 and Meyerholz et al., 2005). In this study in the liver and kidney sections, deposition of amyloid was seen. Infection with Mycobacterium avium subsp. avium was a probable cause of the deposition of amyloid in these pigeons. In necropsy and histopathology examinations of granulomatous lesions no calcification was seen and this finding was consistent with lesions in mycobacterial infections described in birds (Thorel et al., 1997 and Tell et al., 2003). Also in Ziehl-Neelsen’s staining, numerous acid fast bacilli were observed within multinucleated giant cells and in necrotic areas in contrast to other Mycobacterium spp, such as M. bovis and M. tuberculosis, in which organisms are rare within tubercles (Tell et al., 2001). In this study among the examined organs, liver was the best organ for histopathology study of avian tuberculosis because in this organ numerous macroscopic and microscopic granulomatous lesions, acid fast bacilli and amyloid deposition were seen. In conclusion, histopathology findings were typical of avian tuberculosis, including acid fast bacilli and uncalcifiedcaseous necrosis which were surrounded by multinucleated giant cells, macrophages and lymphocytes, also further histopathology studies on other organs of affected pigeons and on other affected bird species are suggested.

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References


بررسی آسیب‌شناسی سل پرندگان در کبودران خانگی به طور طبیعی آلوسه شده با مایکروکاتریوم/ویوم تحت گونه/ویوم

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هدف این مطالعه بررسی آسیب‌شناسی سل پرندگان در کبودران خانگی به طور طبیعی آلوسه شده با ماکروکاتریوم/ویوم تحت گونه/ویوم می‌باشد. سل پرندگان یکی از مهم‌ترین بیماری‌های ماکروکاتریوم/ویوم جنرالس ایجاد می‌گردد. هنگام کشتن از مشخصات کوئی بین 400 تا 600 کوئی بر میلیون نشانه‌های بالینی و شرایط مامایی و سلامتی و نگهداری و سلامت انتخاب کردن و تحت شرایط استاندارد آسان کنی، کالدگذاری و بدنان آن کشتن ماکروکاتریوم/ویوم در حیطه‌های اختصاصی چهت ماکروکاتریوم/ویوم تحت گونه/ویوم صورت گرفت. پس جدایی ماکروکاتریوم/ویوم تحت گونه/ویوم از کبودران سل/ویوم با PCR جدای گردید. همه نماهای آسیب‌های اسید فست جدا شده، به وسیله آزمایش PCR و با پایبرن سل/18S RNA مورد بررسی قرار گرفتند. پس از تشخیص قطعی ماکروکاتریوم/ویوم تحت گونه/ویوم تومور کشتن و آزمایش PCR، مطالعات آسیب‌شناسی بروی 45 نمونه فیسک شده از کبودران میتالی شامل کد، سندگان، پیش میانه، روئدها، کلیدها و منطقه صورت گرفت. مقاطع بافتی طبق روش‌های مداوم تحقیق و توسط روش‌های هم‌اکنون موجود، زیل تلسون و کنگورد (رئین‌آمیوز) ایجاد شدند. بر نوبت پیچیدگی کلودکنشی کی و روهدان، منطقه ارگ‌زنی هم‌بیلارود تنها به همراه با جراحی به‌این‌ها به آزمایش. در این روش‌ها در میان پیرامون میکروکاتریوم/ویوم از آنها انتظار می‌رود که با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم نتواند تولید ناگهانی به‌این‌ها به همراه با جراحی در این روش‌ها میکروکاتریوم/ویوم N