Comparative study of clinical examinations, necropsy findings, and X-ray signs in naturally infected pigeons to avian tuberculosis

Abstract

Mycobacterium avium subspecies *avium* is the most important cause of avian tuberculosis, a chronic disease in many birds. Depending on the infected organs, the clinical manifestations of the disease are usually non-specific and variable. The increasing risk of the disease spreading to the human population has increased the importance of diagnosing this disease in birds. Therefore, the present study investigated avian tuberculosis infection in several lofts of domestic pigeons in Markazi Province, Iran. 12 pigeons from different suspected pigeon lofts were collected, fed a proper ration, and kept under suitable condition. All the pigeons underwent radiographic imaging in lateral and ventrodorsal positions after recording clinical manifestations. Then, they were euthanized and subjected to necropsy examinations. In the necropsy examinations, lesions were sampled, and if no lesion was seen, samples were taken from the liver, then delivered to the tuberculosis reference laboratory and cultured in LJG, LJP, H, and HM culture media. Finally, the grown colonies were tested using Polymerase Chain Reaction (PCR) with the primers of 16S rRNA, IS1245, and IS901. The most prominent clinical manifestations in the infected pigeons included cachexia and articular swelling in wings and legs, while the most common radiographic findings were peri-articular inflammation and renomegaly. Moreover, nodules and lesions in the liver, the gastrointestinal tract, and the abdominal cavity were the most prevalent in necropsy findings. The culture, Ziehl–Neelsen staining results, and PCR test confirmed the infection of all 12 pigeons with *Mycobacterium. avium* subspecies *avium*. Also, the radiographic and necropsy findings showed the destructive effect of avian tuberculosis on the infected pigeons.

Keywords: Avian tuberculosis, *Mycobacterium avium* subspecies *avium*, pigeon, Markazi Province.

1. Introduction

Avian tuberculosis is a critical, chronic, and debilitating disease affecting an extensive range of avian species, including wild and domestic birds, throughout all seasons of the year (1, 2). The related pathogens are *Mycobacterium ovium* and *Mycobacterium genauens* in most cases (3). Depending on the infected organs, the clinical manifestations of the disease are usually non-specific and variable. Infected birds may manifest some clinical symptoms such as diarrhea, cachexia, and pectoral muscle atrophy. In necropsy examination, they showed the development of tuberculosis nodules in internal organs such as the intestines, liver, spleen, and lungs. Infected birds may die within months. However, some in apparently favorable body conditions may experience sudden death due to internal hemorrhage caused by hepatic or spleen blood vessels rupture (1, 4).

As mentioned, infection with Mycobacterium Avium Complex (MAC) does not cause any specific clinical manifestations. Thus, the diagnosis of infection with these pathogens, especially *M. avium* subspecies *avium*, is challenging and should be confirmed using bacterial culture, Ziehl-Neelsen staining, and PCR test (5). The mycobacteria can be transmitted through

contaminated soil, digestion of contaminated water and food, inhalation, and in some cases, cutaneous invasion (1, 6). The birds infected with *the M. avium* subspecies *avium* can survive for an extended time while acting as a carrier, excreting the bacilli in their feces and spreading the infection to other birds and humans. Thus, as the most important source of infection, the feces of infected birds contain numerous bacilli, which can contaminate soil, water, and the environment. According to studies, the bacilli can survive in the soil for 4 years. In addition to birds, *M. avium* can infect other pets and humans (1, 7).

The members of the MAC are classified in Risk Group 2 for humans and should be treated appropriately. They are particularly more dangerous in immunocompromised patients who keep infected birds or eat their undercooked meat (1). Most people infected with *M. avium* subspecies *avium* report keeping domestic birds at home (8). On the other hand, keeping pigeons is quite popular in Iran. Thus, considering the importance of this zoonotic disease and the essential role of birds in its spreading in nature and urban areas, the present study aims to investigate avian tuberculosis in several pigeon lofts in Markazi Province, Iran.

2. Materials and Methods

In the investigations carried out in 10 pigeon lofts in Markazi province, 2 lofts (total of 55 pigeons) suspected of avian tuberculosis were identified. The pigeons of the suspected lofts underwent clinical examinations, and 12 pigeons with clinical manifestations suspected of the disease were selected. The birds were fed a proper ration and kept under suitable conditions for 2 weeks to undergo an acclimatization period for further serological tests, and their clinical manifestations were recorded. Then, all pigeons underwent radiographic imaging using the CR classic (DC-12M, Toshiba, Japan) in lateral and Ventrodorsal (V.D) positions at the veterinary hospital of Tehran University. The suspected birds were euthanized and underwent necropsy, and the findings of necropsy were recorded as well. Moreover, specimens were prepared from the tissues with lesions including; liver, lungs, heart, and gastrointestinal tract, and if no lesion was found, sampling was performed from the liver. The specimens were placed in a screw cap next to dry ice and delivered to the tuberculosis reference laboratory in Razi Vaccine and Serum Research Institute, for culture and definitive diagnosis.

2.1 Culture

A little piece of each specimen was taken using a scalpel and ground in a sterile mortar with sterile sand at the tuberculosis reference laboratory. Then, the homogenized mixtures were decontaminated according to the NALC (N-acetyl-cysteine)-NAOH method (9). The inoculums were cultured on 4 culture slopes including glycerinated Lowenstein-Jensen (LJG) medium, pyruvate-enriched Lowenstein-Jensen medium (LJP), mycobactin J-supplemented Herrold-egg yolk medium (HM) and plain Herrold-egg yolk medium (10). The culture media were kept in an incubator at the temperature of 37° C for 8-10 weeks. Following the formation of the first colonies, Ziehl-Neelsen staining was performed, and the prepared slides were observed using an optical microscope (11).

2.2 PCR

Following staining, the gene extraction was performed using the method by van Soolingen et al. (12). The purity of the specimens was evaluated using the Nano Drop device. Then, they were prepared for PCR, which was performed using the 16srRNA, IS1245, and IS901 primers for detecting mycobacteria, *M. avium*, and *M. avium* subspecies *avium*, respectively. Following electrophoresis, the pattern was photographed using a gel documentation system (Bio-Rad, TX, USA).

3. Results

3.1 Clinical findings

The most common clinical manifestations were cachexia, articular swelling in legs and wings, ocular and periocular involvement, beak deformity, and subcutaneous nodules (Figures 1, 2, 3, and 4; Table 1). Regarding swelling of the joints, the number of wing joint swelling in infected pigeons was higher than that of legs.

Figure 1. Cachectic pigeon, pectoral muscle atrophy.

Figure 2. Tubercular lesions and joint swelling in the leg.

Figure 3. Tubercular lesions and swelling in the wing joint.

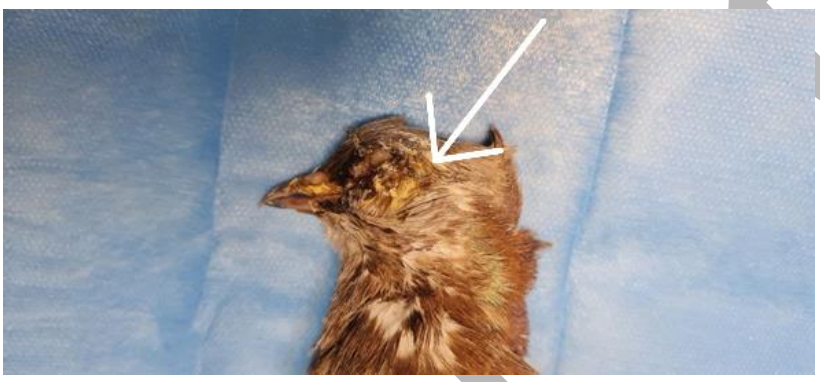

Figure 4. Ocular lesion and periocular nodules.

Table 1. Prevalence of clinical manifestations in the infected pigeons

Clinical manifestations	number (out of 12)	Percentage (%)
Cachexia	11	91.66%
Articular swelling in legs and wings	11	91.66%
Ocular and periocular involvement	7	58.33%
Beak deformity	2	16.66%
Subcutaneous nodule	1	8.33%

3.2 Radiographic findings

The most common radiographic findings were peri-articular inflammation, followed by renomegaly, polyostotic lesions, hepatomegaly, air sacculitis, pneumonia, and gastroenteropathy (Figure 5, Table 2).

Figure 5. Radiographic image of a pigeon suspected of tuberculosis in the V.D position, showing polyostotic lesions and articular swelling in legs and wings.

Table 2. Prevalence of radiographic findings in pigeons suspected with tuberculosis

Radiographic findings	number (out of 12)	Percentage (%)
Peri-articular inflammation	8	66.66%
Renomegaly	7	58.33%
Polyostotic lesions	7	58.33%
Hepatomegaly	6	50%
Air sacculitis	6	50%
Pneumonia	3	25%
Gastroenteropathy	2	16.66%

3.3 Necropsy findings

Necropsy findings included lesions and nodules in the liver, followed by in the gastrointestinal tract, abdominal cavity, heart, and lungs (Figures 6, 7, 8, and 9; Table 3).

Figure 6. Hepatic lesions.

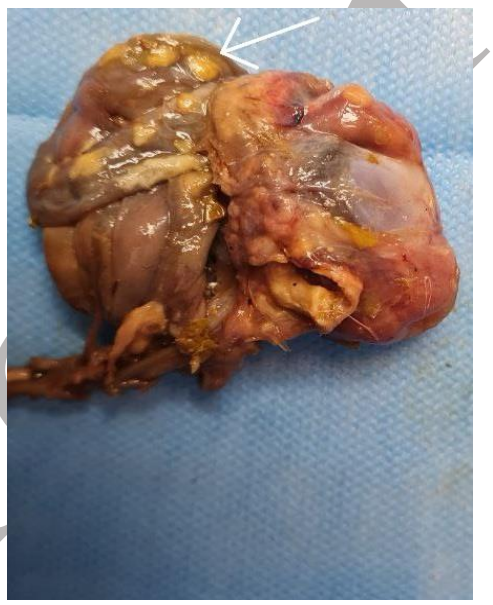

Figure 7. Lesions in the gastrointestinal tract.

Figure 8. Lesions on heart

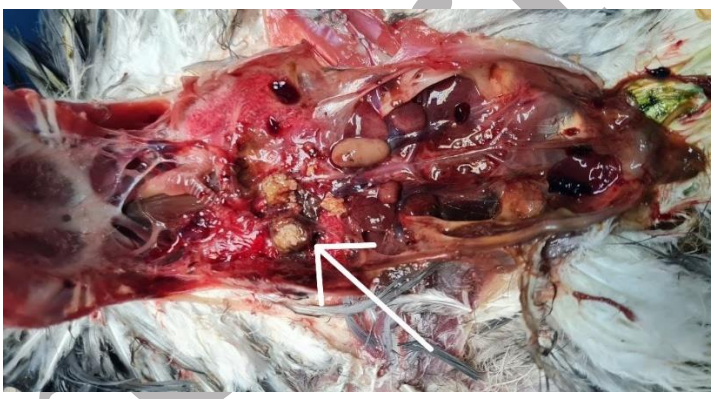

Figure 9. Pulmonary lesions.

Table 3. Prevalence of lesions in necropsy findings in pigeons suspected of tuberculosis.

necropsy findings	number (out of 12)	Percentage (%)
lesions and nodules in the liver	7	58.33%
lesions and nodules in the gastrointestinal tract and abdominal cavity	7	58.33%
Lesions and nodules on the heart	4	33.33%
Lesions and nodules in the lungs	3	25%

3.4 Isolation and molecular identification

Colony growth was observed in all culture media. However, less growth was observed in LP media compared to other culture media. In Ziehl-Neelsen staining all isolates were acid-fast bacilli. These AFB isolates were examined by PCR method using 16S rRNA, IS1245, and IS901 primers for molecular identification. The PCR results confirmed that all the 12 pigeons were infected with the *Mycobacterium avium* subsp. *avium.* (Figures 10, 11, and 12).

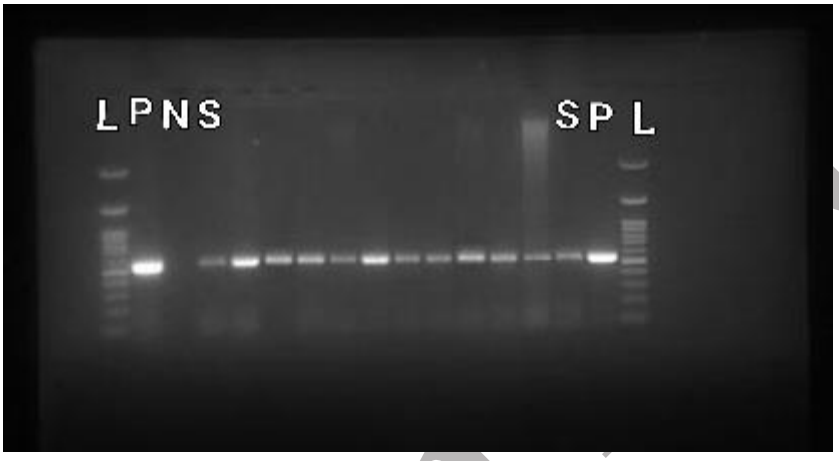

Figure 10. PCR analysis. The 543 bp specific fragment from 16S rRNA primer. From left: ladder (L, DNA size marker 100 base pair ladder), positive control (P, *M. avium* subspecies *avium* strain D4), negative control (N, distilled water), samples (S1-S12), positive control, ladder.

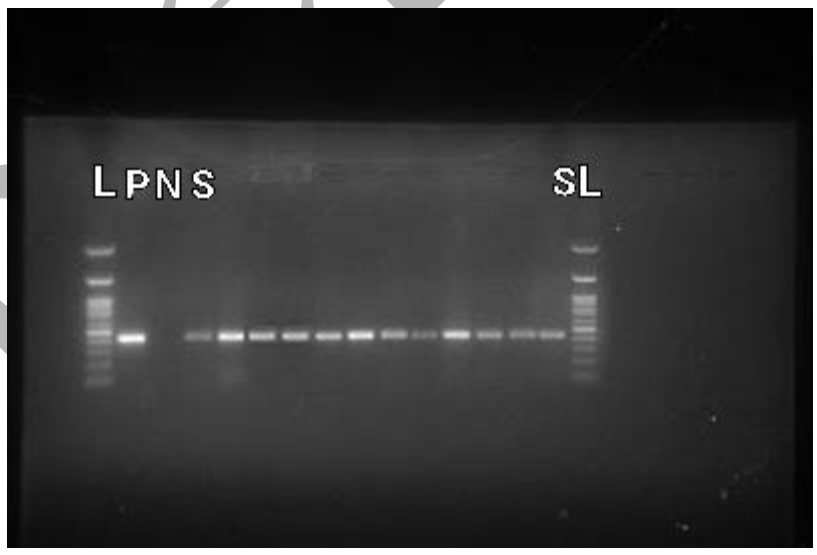

Figure 11. PCR analysis. The 427 bp specific fragment from IS1245 primer. From left: ladder (L, DNA size marker 100 base pair ladder), positive control (P, M. avium subspecies avium strain D4), negative control (N, distilled water), samples (S1-S12), ladder.

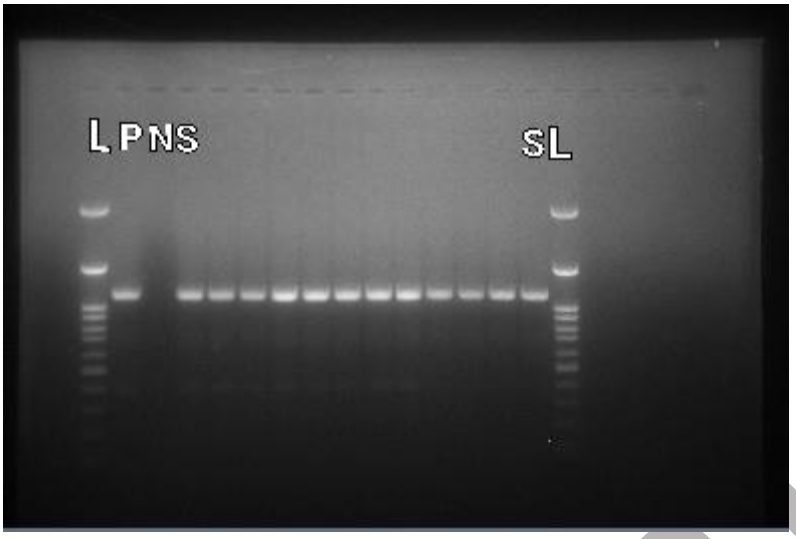

Figure 12. PCR analysis. The 1108 bp specific fragment from IS901 primer. From left: ladder (L, DNA size marker 100 base pair ladder), positive control (P, M. avium subspecies avium strain D4), negative control (N, distilled water), samples (S1-S12), ladder.

4. Discussion

The results of culture, staining, and PCR of the samples taken from all 12 pigeons confirmed their infection with *M. avium* subspecies *avium*. Moreover, the most prominent clinical manifestations in the infected pigeons included cachexia and articular swelling, while the most common radiographic findings were peri-articular inflammation, renomegaly, and polyostotic lesion. Also, the liver and gastrointestinal system had the most lesions and nodules in necropsy findings.

According to our findings, avian tuberculosis can severely involve the gastrointestinal system of infected birds, which is compatible with the previous studies. Several studies have reported that avian mycobacteriosis lesions include yellowish-gray nodules with irregular sizes and shapes that are commonly found in the liver, spleen, and intestines. The ovaries, heart, testes, conjunctiva, skin, lungs, and bone marrow may be involved as well but with less frequency. The present study showed that the birds infected with avian tuberculosis had almost the same necropsy findings and lesion prevalence compared to the other studies (3, 4, 13, 14).

Parvandar *et al*. in their study showed that articular swelling of legs and wings and cachexia were the most prominent clinical manifestations and the liver and gastrointestinal system were the most affected organs in necropsy findings in naturally infected pigeons. All these findings were compatible with the present study (10).

Also, Debelu *et al*. in the study of the epidemiology of avian tuberculosis in selected districts of Oromia region, Ethiopia showed that the liver and gastrointestinal system were the most affected organs. The findings of the mentioned study were compatible with our results regarding the destructive consequences of avian tuberculosis on the gastrointestinal system of pigeons (15).

Moreover, Mayahi *et al*. in a histopathology study of avian tuberculosis in naturally infected domestic pigeons with *Mycobacterium avium* subsp. *avium* showed that most of the granulomatous lesions in the lungs were microscopic and it seems that the lungs were affected more than it was expected. Therefore, in the case of histopathology examination in the current study, more affected lungs may be observed than expected (16).

A study by Jordan *et al*., which was quite similar to the present study, reported the clinical manifestations of gradual weight loss, cachexia, lameness, and diarrhea in the infected birds. Despite the normal appetite in the infected birds, they eventually developed severe cachexia and pectoral muscle atrophy. Moreover, the infected birds died within months. However, some with apparently favorable body conditions experienced sudden death due to internal hemorrhage caused by hepatic or spleen blood vessels rupture. Also, the present study reported cachexia, whether mild or severe, as the most prominent clinical manifestation in 11 out of 12 birds. Thus, the findings of the study by Jordan *et al*. were compatible with the current study about the development of cachexia in infected birds (17).

According to our findings, avian tuberculosis can lead to several different pathological symptoms, and it indicates the very destructive effect of avian tuberculosis in infected pigeons. Considering the zoonotic nature of the disease, it is recommended to monitor all the pigeon lofts and practice biosecurity measurements for its control.

.

Acknowledgment
We would like to express our sincere gratitude to the staff of the Tuberculin Department of Razi vaccine and Serum Research Institute for their kind help and support.

Authors' Contribution
Study concept and design: K.P.A.
Acquisition of data: D.B.K., N.M. and K.P.A.
Analysis and interpretation of data: K.P.A. and D.B.K.
Drafting of the manuscript D.B.K. and K.P.A.
Critical revision of the manuscript for important intellectual content: K.P.A.
Statistical analysis: D.B.K. and K.P.A.
Administrative, technical, and material support: N.M., D.B.K. and K.P.A.
Study supervision. K.P.A

Ethics
The present study was approved by the Ethics Committee of Science and Research Branch, Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC.1401.106).

Conflict of Interest
The authors declare that they have no conflict of interest.

Funding/Support
This work was financially supported by Razi Vaccine and Serum Research Institute, Karaj, Iran.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

References

1. Algammal AM, Hashem HR, Al-Otaibi AS, Alfifi KJ, El-Dawody EM, Mahrous E, Hetta HF, El-Kholy AW, Ramadan H, El-Tarabili RM. Emerging MDR-Mycobacterium avium subsp. avium in house-reared domestic birds as the first report in Egypt. BMC microbiology. 2021 Dec;21(1):1-1.
2. Taira N, Kawasaki H, Takahara S, Chibana K, Atsumi E, Kawabata T. The Presence of Coexisting Lung Cancer and Non-Tuberculous Mycobacterium in a Solitary Mass. Am J Case Rep. 2018 Jun 26; 19:748-751.

3. Dhama K, Mahendran M, Tiwari R, Dayal Singh S, Kumar D, Singh S, Sawant PM. Tuberculosis in birds: insights into the Mycobacterium avium infections. Veterinary Medicine International. 2011 Oct;2011.
4. Tsiouris V, Kiskinis K, Mantzios T, Dovas CI, Mavromati N, Filiousis G, Brellou G, Vlemmas I, Georgopoulou I. Avian Mycobacteriosis and Molecular Identification of Mycobacterium avium Subsp. avium in Racing Pigeons (Columba livia domestica) in Greece. Animals. 2021 Feb;11(2):291.
5. Algammal AM, Wahdan A, Elhaig MM. Potential efficiency of conventional and advanced approaches used to detect Mycobacterium bovis in cattle. Microbial pathogenesis 2019 Sep 1; 134:103574.
6. Kham-Ngam I, Chetchotisakd P, Ananta P, Chaimanee P, Sadee P, Reechaipichitkul W, Faksri K. Epidemiology of and risk factors for extrapulmonary nontuberculous mycobacterial infections in Northeast Thailand. PeerJ. 2018;6: e5479.
7. Kindu A, Getaneh G. Prevalence of avian tuberculosis in domestic chickens in selected sites of Ethiopia. J Vet Sci Technol. 2016;7(377):2.
8. Babacan O, Bülent BA, SAREYYÜPOĞLU B. PCR detection of Mycobacterium genavense DNA in fecal samples of caged birds. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2020 Mar 3;67(2):201-4.
9. Pfyffer GE, Brown-Elliott BA, Wallace Jr RJ. Mycobacterium: general characteristics, isolation, and staining procedures. Manual of clinical microbiology. 2003; 1:532-59.
10. Parvandar-Asadollahi K, Mosavari N, Mayahi M. Genotyping of Mycobacterium avium subsp. avium isolates from naturally infected lofts of domestic pigeons in Ahvaz by IS901 RFLP. Iranian journal of microbiology. 2015 Oct;7(5):260.
11. Tell, LA.; Foley, J.; Needham, ML.; Walker, RL. (2003). Diagnosis of avian mycobacteriosis; comparison of culture, acid-fast stains, and polymerase chain reaction for the identification of Mycobacterium avium in experimentally inoculated Japanese quail (Coturnix coturnix japonica). Avian Diseases, (47): 444-452.
12. Huard, R.C., de Oliveira Lazzarini, L.C., Butler, W.R., van Soolingen, D. and Ho, J.L., 2003. PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. Journal of clinical microbiology, 41(4), pp.1637-1650.
13. Casaubon Huguenin M.T., Brugère Picoux J. Bacterial Diseases. In: Brugère Picoux J., Vaillancourt J.P., Bouzouaia M., Shivaprasad H.L., Venne D., editors. *Manual of Poultry Diseases.* AFAS; Paris, France: 2015. pp. 360–363.
14. Kriz P., Slana I., Kralik P., Babak V., Skoric M., Fictum P., Docekal J., Pavlik I. Outbreak of *Mycobacterium avium* subsp. *avium* infection in one flock of domestic pigeons. *Avian Dis.* 2011; 55:503–508.
15. Debelu T, Abunna F, Mamo Kassa G, Ameni G. Epidemiology of Avian Tuberculosis in Selected Districts of Oromia Region, Ethiopia. Vet Med Int. 2022 Jan 27; 2022:6933701.
16. Mayahi M, Mosavari N, Esmaeilzadeh S, Parvandar Asadollahi K. Histopathological study of avian tuberculosis in naturally infected domestic pigeons with Mycobacterium avium subsp. avium. Iranian Journal of Veterinary Science and Technology. Vol. 5, No. 1, 2013, 45-56

17. Jordan F.T.W., Hampson D.J. Some other bacterial diseases. In: Pattison M., Mc Mullin P.F., Bradbury J.M., Alexander D.J., editors. Poultry Diseases. 6 th ed. Elsevier Limited; Philadelphia, PA, USA: 2008. pp. 250–254.