Diagnosis of Avian Tuberculosis in laying hens by pathological, microbiological and polymerase chain reaction (PCR): Case report

ABSTRACT

Avian tuberculosis was described at 50 week-old Lohmann chickens in a commercial chicken farm. The study materials were 50 week-old 20 pieces of chicken from 2 coops and 16 week-old 20 pieces of pullets from 4 coops in a commercial chicken farm. After necropsy, samples were processed routinely for histopathological and microbiological examinations. Macroscopically, hard consistency, numerous and different sizes, whitish-yellow caseificated-calcifiated nodules were seen on the liver, spleen, kidneys and intestinal serosa. In the microscopic examination, various sized granulomas, which have been caseification necrosis surrounded by epithelioid histiocytes and multinucleated giant cells, were found at liver, spleen, wall and serosa of intestine. Numerous acid-fast bacteria were seen on histopathology at necrosis and macrophages in the liver, spleen and the intestines by Ziehl – Neelsen staining in all cases. Mycobacterium avium spp was produced at microbiological inoculations in liver, spleen, intestines and ovaries. Mycobacterium avium subsp. avium from these cultures was identified by PCR. It was thought that infection could be by fecal-oral route due to both intestinal tuberculosis in hens and common disease in coop. Therefore, the role of chicken manure may also be taken into account for the spreading of the disease.

Keywords: Hens, Mycobacteriosis, Pathology, PCR

INTRODUCTION

Avian tuberculosis is a chronic infectious disease characterized by low productivity and weakening caused by *Mycobacterium avium* (MA) (Fulton and Thoen, 2003; Jordan and Hampson, 2008). The disease has caused major losses to hen populations. It is not only seen predominantly in laying hens, but has also been detected in pigeons, turkeys, parrots, pheasants, waterfowl and wild birds (Bolfion et al., 2010; Cromie et al., 1993; Gonzalez et al., 2002; Gümüşsoy et al., 2006; Kapakin and Alçığır, 2009; Keymer et al., 1982; Kriz et al., 2010; Kul et al., 2005; Kutsal and Sağlam, 1988; Mayahi et al., 2013; Prukner-Radovcic et al., 1998; Saggese et al., 2007; Sezen et al., 1986; Sousa et al., 2008). Transmission is carried with digestion of contaminated feed and water. It has been reported that inhalation is not an effective method of contracting the infection (Fulton and Thoen, 2003). MA can be isolated from the egg in a natural infection, but it failed to create avian tuberculosis in hatched chicks. The agents die after boiling of eggs for 6 minutes (Fulton and Thoen, 2003). The most common symptom is increasing weakening of sick birds. Greenish diarrhea and deaths occur in the chronic period.

How to cite this article
Yavuz, O., Özdemir, Ö., Sayın Z., Hatipoğlu, F., Hadimli, HH. (2021). Diagnosis of Avian Tuberculosis in laying hens by pathological, microbiological and polymerase chain reaction (PCR): Case report. *Journal of Advances in VetBio Science and Techniques*, *6*(3), 312-317. https://doi.org/10.31797/vetbio.935334

Case Report

Orhan Yavuz[1a]
Özgür Özdemir[2b]
Zafer Sayın[3c]
Fatih Hatipoğlu[2,4d]
Hasan Hüseyin Hadimli[3e]

[1]Department of Pathology, Faculty of Veterinary Medicine, Aksaray University, Aksaray
[2]Department of Pathology, Faculty of Veterinary Medicine, Selçuk University, Konya
[3]Department of Microbiology, Faculty of Veterinary Medicine, Selçuk University, Konya
[4]Department of Pathology, Faculty of Veterinary Medicine, Kyrgyzstan-Turkey Manas University, Bishkek, Kyrgyzstan

ORCİD-
[a]0000-0002-8075-9564
[b]0000-0002-1595-0557
[c]0000-0001-6096-4440
[d]0000-0002-0103-5868
[e]0000-0002-7665-687X

Correspondence
Orhan YAVUZ
vetorhanyavuz@gmail.com

Article info
Submission: 31-05-2021
Accepted: 07-11-2021

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
• http://dergipark.org.tr/vetbio

The most affected organs are liver, spleen, intestine, bone marrow and lung. Numerous large and small, hard structured, whitish-yellow nodules are observed in affected organs during the necropsy. There are typical tubercles in microscopic examination (Fulton and Thoen, 2003). A large number of acid-fast bacteria can observe by Ziehl – Neelsen (ZN) staining. Diagnosis of avian tuberculosis in hens depends on the demonstration of MA in live or dead chickens, tuberculin test or serological and PCR techniques (OIE, 2014). MA causes zoonotic infections in humans especially in immunocompromised individuals such as leukemia patients or infected with HIV. MA can lead to generalized mycobacteriosis in such humans, but healthy humans have a low susceptibility to MA infection (Coelho et al., 2013).

CASE DESCRIPTION

In this study, an outbreak of avian tuberculosis was identified on a commercial chicken farm by clinical, pathological findings and PCR techniques. The study materials were 20 pieces of 50-week-old chickens from two coops and 20 pieces of 16-week-old pullets from four coops in a commercial chicken farm with 400 thousand chicken capacity in Turkey. All animals were brought dead. The farm exhibited fine biosecurity conditions and hens fed with commercial feed. Diets have also included meat and bone meal. An increase in number of deaths (up to 3%) was observed over 2 months period in the farm. Clinically, weakening and decrease of egg production were noticed in hens and pullets. The rate of egg production was decreased to 80% from 100% in the farm.

All chickens were necropsied and examined grossly. Tissue samples were fixed in 10% formalin for 24 hours and processed routinely, then embedding in paraffin. Embedded tissue samples were cut at 5µm thick and stained with Hematoxylin & Eosin (HE) and ZN and examined with light microscope. Smears were stained with ZN for cytological examination.

Samples were inoculated into Lowenstein-Jensen culture medium for microbiological examination. Isolation and identification of *Mycobacterium avium subsp. avium* (MAA) isolates were made using the protocol described by Ambrosio et al. (2008). Specimens were cultured on Lowenstein-Jensen medium at 37°C for 6 weeks and isolates were stored at -80°C. Chromosomal DNA of MAA was extracted using the protocol provided in Promega Wizard Genomic DNA purification Kit (Cat No: A1120). The DNA samples were stored at -20°C until used as templates for amplification. MAA specific primer pairs were used in polymerase chain reaction (PCR) for amplification of the insertion sequence IS901 of MAA as described by Kunze et al. (1991). The sequence of forward primer was 5'- GCA ACG GTT GTT GCT TGA AA-3' and reverse primer was 5'-TGA TAC GGC CGG AAT CGC GT-3'. Specific primers showed a single amplicon of 1108 bp. The extracted DNA was amplified in a total volume of 50 µL (5 µL 10× PCR buffer, 250 mM from each dNTPs (MBI Fermentas), 1.25 U taq polymerase (MBI Fermentas), 0.5 µM each of primers (IDT), 1.5 mM MgCl2, and 5 µL extracted DNA. The cycling conditions with the Techne Progene (Cambridge Ltd., UK) were the initiation step at 94 ˚C for 3 min, followed by 33 cycles coupling 94 ˚C for 1 min, 66 ˚C for 45 s and 72 ˚C for 4 min and a final extension period at 72 ˚C for 3 min (14). Ten µL of amplification products were submitted to electrophoresis in 2% agarose gel in Tris–borate EDTA buffer and the 100 bp ladder DNA marker (MBI Fermentas) was run concurrently. The ethidium bromide-stained DNA bands were visualized and the gel photographed.

There was no difference in terms of lesion location and severity between 16 and 50 week

old hens in the farm. Macroscopically, a great number of large and small, hard consistency, whitish-yellow, calcified cut surfaces of nodules were found in the liver (Figure 1A.), spleen (Figure 1B.), kidneys, serosa of the intestines (Figure 1C.) and ovaries (Figure 1D.).

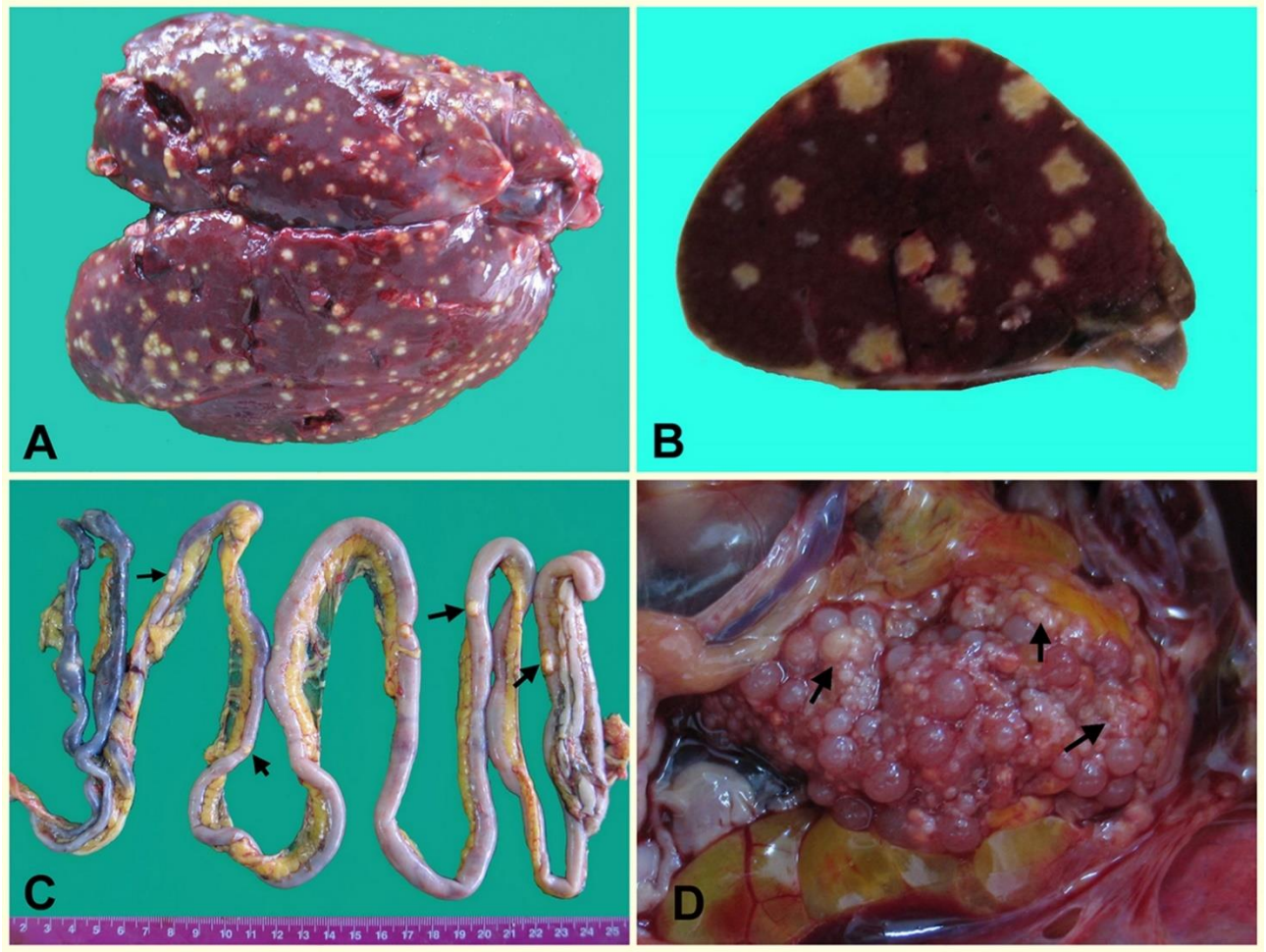

Figure 1. A. A great number of large and small, hard structured, whitish-yellow tubercles in the liver. **B.** Cut surfaces of spleen. **C.** Serosa of the intestines (arrows). **D.** Ovaries (arrows)

Various sized granulomas (Figure 2A.), which have been identified as caseification necrosis surrounded by epithelioid histiocytes and multinucleated giant cells (Figure 2B.), were found in liver, spleen, wall and serosa of intestine at microscopical examination. It was noticed that granulomas in the serosa caused ulcers when it came to mucosa. Numerous acid-fast bacteria were determined at necrosis areas by cytological smears (Figure 2C.) and histopathology, as well as macrophages in the liver, spleen and intestines by ZN staining. The agent was identified as a MAA by PCR analysis on microbiological inoculations of liver, spleen, intestines, lung and ovaries (Figure 2D.). There were no findings related to tuberculosis in the other coops of same farm.

DISCUSSION

It has been reported that MA infection is commonly observed in pigeons, captive pets and wild birds in the zoo (Bolfion et al., 2010; Gümüşsoy et al., 2006; Kapakin and Alçığır, 2009; Kriz et al., 2010; Mayahi et al., 2013; Prukner-Radovcic et al., 1998; Sezen et al., 1986; Sousa et al., 2008). However, rarely are outbreaks observed on commercial chicken farms under inadequate hygiene conditions (Gonzalez et al., 2002). Atypical symptoms such as chronic period of disease, cachexia, muscle atrophy and decreased egg production complicates the clinical diagnosis. In such cases, pathological findings are helpful for diagnosis as

well as identification of agents by advanced molecular techniques (OIE 2014).

Gonzalez et al. (2002) mentioned two different models for disease as clinical and pathologically. In the first type generalized tuberculosis was observed as a result of cachexia and decreased productivity. Secondly, there were limited infraorbital sinus lesions and no loss of egg production. Clinically, cachexia, weight loss and decreased egg production have been observed in our case. Grossly generalized tuberculosis lesions were observed on internal organs such as the liver, spleen and intestines with no evidence of macroscopic lesions in infraorbital sinuses and the lungs. This situation was interpreted as primarily lesions occurring in organs such as the liver and intestines due to oral transmission.

The agents were identified in the liver, spleen, intestines, ovary and the lungs by PCR analysis with IS 901 primer. Despite there being no macroscopic or microscopic findings in the lungs, MAA was identified by PCR. Thus, it is thought to be the causative agent generalized to the lungs from the digestive organs.

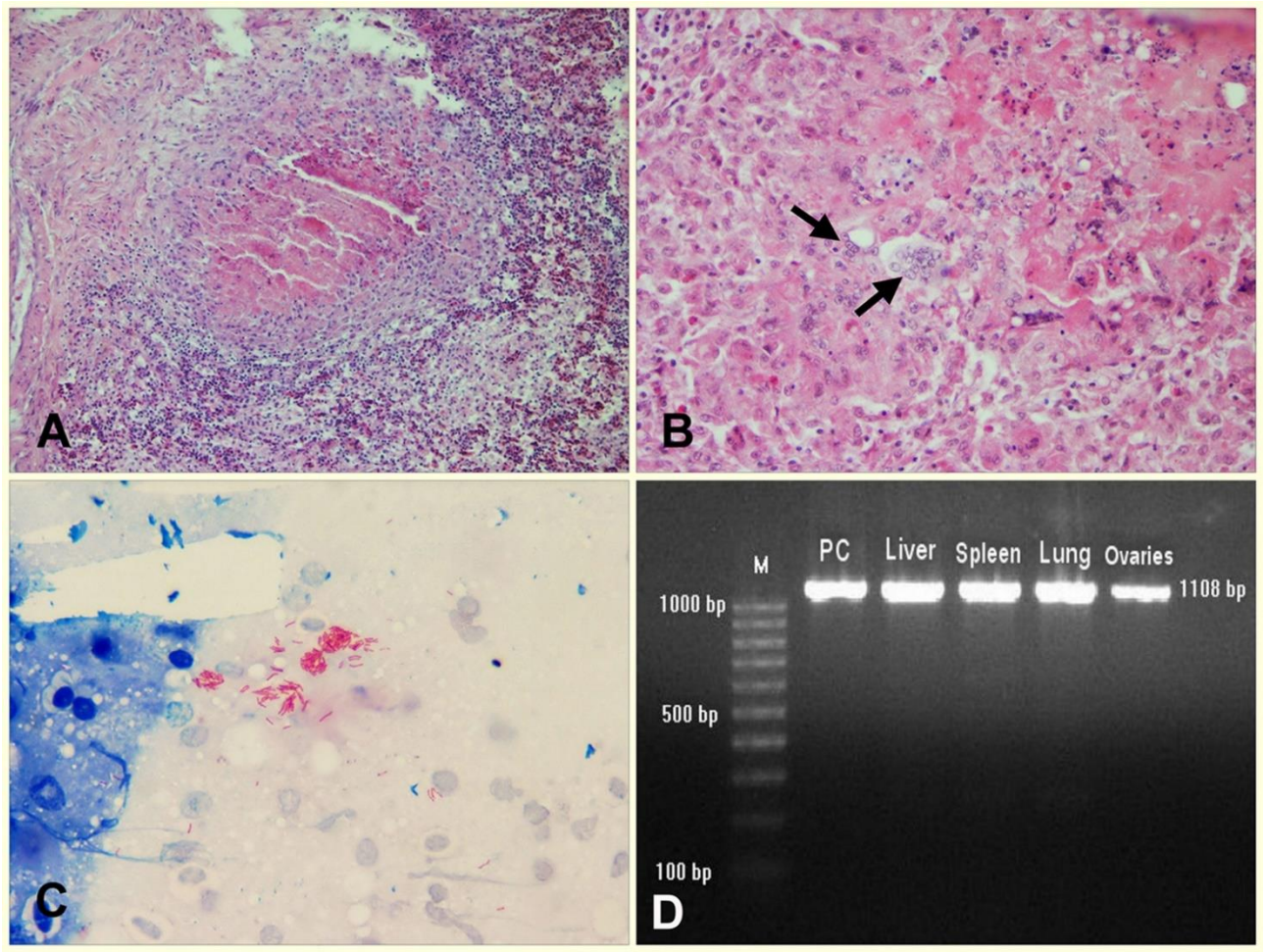

Figure 2. A. Caseified tubercles which have been surrounded by mononuclear, epithelioid and giant cells, **Spleen,** x200, HE, **B.** Multinucleotid giant cells in the granulomas (arrows), **Liver,** x400, HE, **C.** Acid-fast bacteria in the cytological examinations, **Liver**, x1000, ZN, **D.** The image of agarose gel electrophoresis of the MAA culture positive samples by IS 901 primer **(M:** Fernentas 100 bp DNA marker SM 0321, **PC:** Positive control- *M. Avium* ATCC 25291).

Mycobacterium avium can be isolated from the egg in natural infection, however the hatched chickens failed to develop avian tuberculosis (Fulton and Thoen, 2003). Although the MA were isolated and identified from the ovaries of infected animals, there was no causative agent detected from eggs of these hens. It has been expressed that *Mycobacterium avium* are not always identified from eggs. They may vary with

intensity and severity of the infection (Shitaye et al., 2008).

Although Fulton and Thoen (2003) reported to avian tuberculosis is less prevalent in young chickens, extensive and diffuse tuberculosis was observed in this case report. The occurrence of fecal-oral contamination due to a large amount of agents entering to the body via oral route and the presence of lesions in the intestines have shown that increase the prevalence of the disease in the farm. The presence of extensive intestinal lesions in these hens supports this possibility. Besides, recently informed from farm veterinarian that the meat and bone meal join to the diet, raised concerns about feeding. Therefore, it has been expressed that such hens are an important source of spreading the disease. It was estimated that hens might be infected with tuberculosis orally from feaces, because Hejlicek and Treml (1995) emphasized that the contaminated feaces of infected birds are the major source of infection for other hens.

In conclusion, it was thought that infection could have been by fecal-oral route due to the presence of intestinal tuberculosis in hens. Therefore, the role of chicken manure may also be taken into account for the spreading of the disease.

ACKNOWLEDGMENT

It was presented as a poster presentation in the VI. National Veterinary Pathology Congress.

Ethical approval: Procedures performed with dead animals or their tissues, slaughterhouse materials and waste fetuses are not subject to the permission of the Animal Experiments Local Ethics Committee".

Conflict of interest: The authors declared that there is no conflict of interest

KAYNAKLAR

Ambrosio, S.R., Márcia De Deus, O.E., Rodriguez, C.A.R., Neto, J.S.F., & Amaku, M. (2008). Comparison of three decontamination methods for *Mycobacterium bovis* isolation. *Brazilian Journal of Microbiology*, 39, 241-4.

Bolfion, M., Salehi, M., Ashrafi, H.J., Soleimani, K., Keshavarz, R., Aref, P.R., Mohammad, T.M., Tadayon, K., & Mosavari, N. (2010). Outbreak of avian Mycobacteriosis in flocks of domestic pigeons: an epidemiological approach. *Iranian Journal of Microbiology*, 2, 189-93.

Coelho, A.C., Pinto, M.L., Matos, A., Matos, M., & Pires, M.A. (2013). Mycobacterium avium complex in domestic and wild animals. In: R.P. Carreira (Eds). *Insights from Veterinary Medicine* (pp. 91-128). Rijeka: InTech Publishing. https://doi.org/10.5772/56196

Cromie, R.L., Brown, M.C., Forbes, N.A., Morgan, J., & Stanford, J.L. (1993). A comparison and evaluation of techniques for diagnosis of Avian Tuberculosis in wildfowl. *Avian Pathology*, 22, 617-30.

Fulton, M.R., & Thoen, C.O. (2003). Tuberculosis. In: Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. Mcdougald, D.E. Swayne (Eds). *Diseases of poultry. 11th ed.* (pp. 836-844). Iowa State University Press.

Gonzalez, M., Rodriguez, A.B., Gimeno, I., Flores, J.M., & Pizarro, M. (2002). Outbreak of avian Tuberculosis in 48 week-old commercial layer hen flock. *Avian Disease*, 46, 1055-61.

Gümüşsoy, K.S., Beyaz, L., Aydin, F., Özcan, M., & Atasever, A. (2006). Avian Tuberculosis in Kayseri zoo. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi*, 3, 25-8.

Hejlicek, K., & Treml F. (1995). Comparison of pathogenesis and epizootiology signification of avian mycobacteriosis in different sorts of domestic and free living synanthropic fowl. *Veterinární Medicína*, 40, 187–94.

Jordan, F.T.W., & Hampson, D.J. (2008). Mycobacterium Avium – Avian Tuberculosis. In: M. Pattison, P.F. Mcmullin, D.J. Alexander (Eds). *Poultry disease. 8th ed.* (pp. 250-254). Saunders Elsevier Press.

Kapakin, K.A.T., & Alçiğir, G. (2009). The case of Tuberculosis in a pigeon. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 15, 477-9.

Keymer, I.F., Jones, D.M., Pugsley, S.L., Wadsworth, P.F. (1982). A survey of tuberculosis in birds in the regent's park gardens of the zoological society of London. *Avian Pathology*, 11, 563-9.

Kriz, P., Slana, I., Mrlik, V., Moravkova, M., Kralova, A., Krizova, K., & Pavlik, I. (2010). *Mycobacterium avium* subsp. *avium* in domestic pigeons (*Columba livia* f. *domestica*) diagnosed by direct conventional multiplex PCR: a case report. *Veterinární Medicína,* 55, 87-90.

Kul, O., Tunca, R., Hazıroğlu, R., Diker, K.S., & Karahan, S. (2005). An outbreak of avian Tuberculosis in peafowl (*Pavo cristatus*) and pheasants (*Phasianus colchicus*) in a zoological aviary in Turkey. *Veterinární Medicína,* 50, 446-50.

Kunze, Z.M., Wall, S., Appelberg, R., Silva, M.T., Portaels, F., & Mcfadden, J.J. (1991). IS901, a new member of a widespread class of atypical insertion sequences, is associated with pathogenicity in *Mycobacterium avium*. *Molecular Microbiology*, 9, 2265–72.

Kutsal, O., & Sağlam, M. (1988). Güvercinlerde tüberküloz olgularının değerlendirilmesi. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* 35, 545-52.

Mayahi, M., Mosavari, N., Esmaeilzadeh, S., & Asadollahi, K.P. (2013). Avian Tuberculosis in naturally infected lofts of domestic pigeons, isolation, molecular identification and study of necropsy findings. *The International Journal of Applied Research in Veterinary Medicine*, 11, 194-201.

OIE. (2014, December 20). *Avian Tuberculosis. OIE Terrestrial Manual 2014. Chapter 2.3.6.* http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.06_AVIAN_TB.pdf

Prukner-Radovcic, E., Culjak, K., Sostaric, B., Mazija, H., & Sabocanec, R. (1998). Generalized Tuberculosis in pheasants at a commercial breeding farm. *European Journal of Wildlife Research*, 44, 33-9.

Saggese, M.D., Riggs, G., Tizard, I., Bratton, G., Taylor, R., & Phalen, D.N. (2007). Gross and microscopic findings and investigation of the aetiopathogenesis of mycobacteriosis in a captive population of white-winged ducks (*Cairina scutulata*). *Avian Pathology*, 36, 415-22.

Sezen, Y., Erer, H., & Erganiş, O. (1986). Bir güvercinde tüberküloz olgusu. *Eurasian Journal of Veterinary Science*, 2, 163-6.

Shitaye, J.E., Matlova, L., Horvathova, A., Moravkova, M., Dvorska, L., Trcka, I., Lamka, J., Treml, F., Vrbas, V., & Pavlik, I. (2008). Diagnostic testing of different stages of avian Tuberculosis in naturally infected hens (*Gallus domesticus*) by the tuberculin skin and rapid agglutination tests, faecal and egg examinations. *Veterinární Medicína,* 53, 101-10.

Sousa, E., Momo, C., Costa, T.P., Pinheiro, S., & Werther, K. (2008). Disseminated avian Tuberculosis in captive *Ara macao*. *Brazilian Journal of Veterinary Pathology*, 1, 28-31.