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**Khaled Kaboudi**

Department of Poultry Farming  
and Pathology – National  
Veterinary School of Tunisia

**Abdelkader Amara**

Department of Pathological  
Anatomy and Histology –  
National Veterinary School of  
Tunisia

**Moncef Bouzouaia**

Department of Poultry Farming  
and Pathology – National  
Veterinary School of Tunisia

## Avian tuberculosis in a backyard poultry flock in Tunisia: Case report

**Khaled Kaboudi, Abdelkader Amara and Moncef Bouzouaia**

### Abstract

Tuberculosis is a chronic infectious disease affecting several species of domestic and wild birds. It is due to the infection of the gastrointestinal tract, liver and spleen by various *Mycobacterium* species. This disease is more commonly seen in zoos and wild avian species (Del Pilar Silva *et al.*, 2009) [5]. *Mycobacterium avium* (serotypes 1, 2 and 3) and *Mycobacterium genavense* represent the most incriminated germs in the case of avian tuberculosis. This topic describes the observations of the first report of a typical case in a chicken issued from a backyard flock received at the avian clinic of the National Veterinary School of Sidi Thabet. This case prompted-us to bring and develop pathological aspects.

**Keywords:** tuberculosis, backyard poultry, histology, Tunisia

### 1. Introduction

Tuberculosis is a chronic infectious disease affecting several species of domestic and wild birds. Caused by the proliferation of different *Mycobacterium* species in the digestive tract, the liver and the spleen, this infection is mostly observed in zoological parks and in wild avian species (Del Pilar Silva *et al.*, 2009 ; Stepień-Pyśniak *et al.*, 2016 ; Zhu *et al.*, 2016) [5, 18, 19]. *Mycobacterium avium* (serotypes 1, 2 and 3) and *Mycobacterium genavense* are the most incriminated pathogens in avian tuberculosis. Spread of the infection to human is possible, particularly in immuno-depressed patients.

Hejlícek and Treml (1995) [11] classified sensitive avian species to experimental infection by *Mycobacterium avium* in four groups: very sensitive species (domestic fowl, sparrow, pheasant, partridge and seagull), little sensitive species (guinea fowl, turkey), resistant species (goose, duck) and very resistant species (pigeon, turtledove and crow).

Infected birds are considered as the most important source of germ, which is issued from ulcerative lesions of intestines and excreted in feces to contaminate environment. *Mycobacterium*, such as *M. avium*, can resist during several years (more than 4 years) which can explain the reappearance of the disease in the same farm, even after elimination of all birds. Resistance of these germs is also considerable in variable temperatures. They can survive about 168 days to 244 days at 20°C and 37°C, respectively (Shalk *et al.*, 1935; Schliesser and Weber, 1973) (in Fulton and Thoen, 2003) [6]. *M. avium* was isolated from incubated eggs of naturally infected breeder hens. However, correspondent chicks do not develop the disease. Dissemination of the bacteria can be assured by poultry infected carcasses.

The aim of this article is to report and develop, for the first time, the anatomo-pathological aspects of a typical case of tuberculosis in backyard layer hen, diagnosed in the Avian Clinic of the National Veterinary School of Sidi Thabet (Tunisia).

### 2. Materials and Methods

#### 2.1. Case history

Sporadic mortality was appeared in a free-range poultry flock, composed of 20 birds, with different provenance and age, reared outdoor. The 18 months old dead layer hen was very lean. It presented an allowance and a whitish diarrhea before death. Biosecurity measures in this little farming are poor, or even absent.

**Correspondence**

**Khaled Kaboudi**

Department of Poultry Farming  
and Pathology – National  
Veterinary School of Tunisia

The procurement is based on the purchase of unknown provenance chickens.

## 2.2. Gross pathology and bacteriology analysis

The autopsy, was performed at the clinic of Avian Pathology of The National Veterinary School of Sidi Thabet (Tunisia). A Ziehl-Neelsen coloration was performed on different damaged organs.

## 2.3. Histopathological study

Histopathological analyzes were performed at the Histology department of the National Veterinary School of Sidi Thabet (Tunisia). Organ Harvesting (liver, spleen, intestines, pancreas, kidneys) are immediately emerged in a 10% formalin solution in order to fix them. After processing sections according to conventional methods of histological techniques, two types of stains are made, hematoxylin-eosin (HE) and the Ziehl-Neelsen.

## 3. Results

### 3.1. Gross pathology and bacteriology

The necropsy exam revealed during the external examination, weight loss and traces of white diarrhea. At the opening of the thoraco-abdominal cavity, the liver appears enlarged, greenish-brown color and dotted with gray-yellowish nodules of variable size (2 to 5 mm), which are detected also within the organ. (Figure 1)



**Fig 1:** enlarged liver sprinkled with caseous nodules

These nodules are observed in the other viscera, mainly spleen, intestine, mesentery, gizzard, pancreas and kidneys (Figures 2 and 3). The Ovary appears deformed and atrophied.



**Fig 2:** Splenomegaly with multiple caseous nodules on the spleen



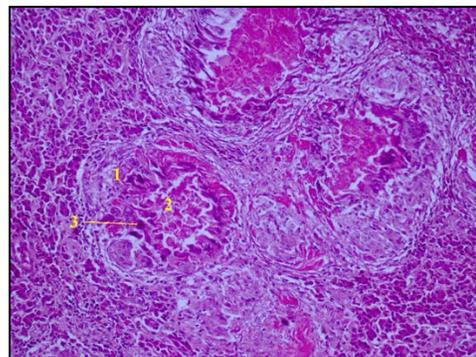
**Fig 3:** Multiple caseous nodules with different sizes on the intestines, the mesentery and the gizzard

Several diseases are discussed on the basis of the necropsy results, particularly tuberculosis, Hjarre disease (coligranulomatosis), lymphoid leukemia and visceral form of Marek's disease.

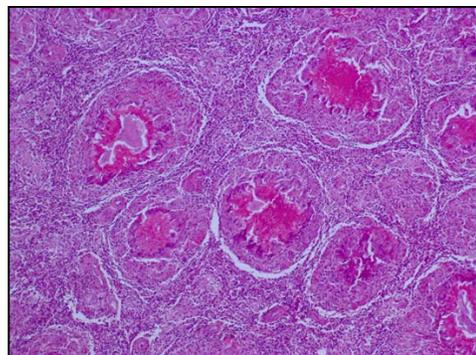
The presence of clusters of acid-fast bacilli is thus confirmed.

### 3.2. Histopathology

This study showed the presence of immunological granulomas (Koster follicles) in various tissues and viscera. Indeed, liver, spleen, intestines, kidney and pancreas are the site of multiple granulomas centered on an acidophilic disintegration necrosis range, surrounded by a macrophage and epithelioid reaction, with many small and irregular Langhans giant cells. The periphery was occupied by a fibro-connective hull and a few lymphocytes (Figures 4 and 5).

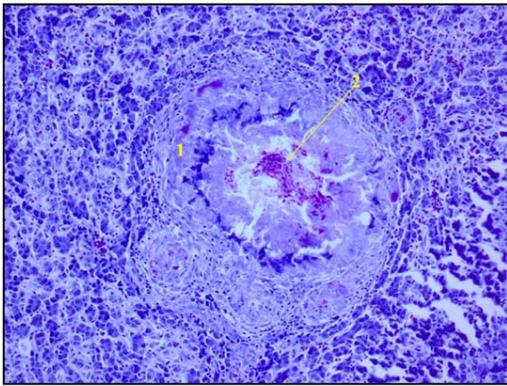


**Fig 4:** Liver. Koester follicle (1) centered on a necrosed foci (2) and surrounded by épthélioide cells and Langhans giant cells (3) (HEx200)

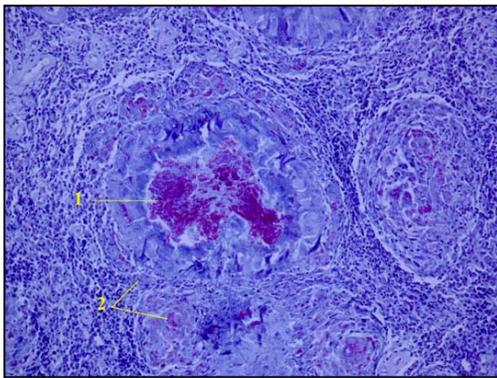


**Fig 5:** Spleen. Multiple Koester follicles in the spleen (HEx100)

Clumps of acid-fast bacilli were noted in Ziehl-Neelsen stained sections. These bacilli were accumulated in the center of the granulomas and in the cytoplasm of macrophages (Figures 6 and 7).



**Fig 6:** Liver. Koester follicles (1) and piles of acid-fast bacilli in the center (2) (ZN x 200)



**Fig 7:** Spleen. Acid-fast bacilli in the center of Koester follicle (1) and the cytoplasm of macrophages (2) (ZN x 400)

#### 4. Discussion

Absent in commercial poultry in Tunisia, avian tuberculosis is rather observed in free-range flocks, where it is diagnosed in fowl, turkey, pheasant and, more rarely in goose and duck.

Several risk factors can favor the emergence of the disease in backyard poultry breeding. Indeed, unknown provenance of animals (local markets, other farms), mixture of avian and mammalian species in the same flock, possible contact between domestic poultry and wild birds and poor level of biosafety measures are considerable risk factors in the introduction and development of mycobacterium species in the free-range flocks (Hejlíček and Tremel, 1993; Dhama *et al.*, 2008; Kriz *et al.*, 2013; Abda *et al.*, 2015) [1, 4, 10, 12].

Lesions caused by *M. avium* may be localized or generalized in mammals, including cattle, rabbits and horses often present on farms in contact with infected birds (Lesslie and Birn, 1967; Gunnes *et al.*, 1995) [8, 14]. The germ can multiply in different tissues for a long time making the animal susceptible to any tuberculin.

Clinical signs of avian tuberculosis are not pathognomonic. The infected animal passes through three phases: a latency phase, a development phase of lesions and finally phase of cachexia. The ingestion of the tuberculosis bacillus causes an intestinal infection and promotes bacillemia. The liver is among the first organs affected, then the germ spreads to the other tissues.

Avian tuberculosis is rarely observed in young birds, which are not necessarily more resistant to infection, but the disease is more common in adults. Indeed, the disease gradually settles over a long period. The lesions of avian tuberculosis are often less severe in young chickens than in older birds.

Gross lesions described in our study were similar to those revealed by many authors in different bird species naturally infected by avian tuberculosis. In this way, lesions observed in the liver, the spleen and the intestines were in agreement with the results of Randall and Reece (1996) [17], Gonzalez *et al.* (2002) [7], Mayahi *et al.* (2013) [16], Majeed *et al.* (2014) [15] and Zhu *et al.* (2016) [19].

However, contrarily to the descriptions of Beytut *et al.* (2001) [2] and Gonzalez *et al.* (2002) [7], no lesions were identified in the lungs and in the heart and the infra-orbital sinus, respectively. In addition, we have not detected lesions in the conjunctiva and the joints as was described by Mayahi *et al.* (2013) [16] on pigeons affected naturally by tuberculosis. (Table 1)

**Table 1:** Comparative results of lesions location in natural cases of avian tuberculosis in birds

Bird	Liver	Spleen	Intes.	Pancr.	Kidney	Gizzard	Lung	Heart	Loco.	Conj.	Sinus	Authors
BC	+	+	+	+	+	+	-	-	-	-	-	Our study
LH	+	+	+	-	-	-	-	+	-	-	+	Gonzalez <i>et al.</i> (2002) [7]
LH	+	+	+	-	-	-	+	-	-	+	-	Beytut <i>et al.</i> (2001) [2]
Pigeon	+	+	+	-	-	+	-	+	+	-	-	Mayahi <i>et al.</i> (2013) [16]
Pheasant	+	+	+	-	-	-	-	-	-	-	-	Kul <i>et al.</i> (2005)

BC: Backyard chicken; Conj. : Conjunctiva; LH: Layer hen; Intes. : Intestine; Loco. : Locomotor system; Pancr. : Pancreas

Inflammatory reaction noted in the intestine, related to the tuberculous granulomas, could explain the malabsorption and the disturbance of the digestive tract function, explaining growth retardation, weight loss and decrease in production. Histopathological lesions were those of a tuberculosis. They were characterized by the presence of multiple follicles of Koester with different size. Our observations were similar to those of many authors. Besides the examined viscera in this study, tuberculosis lesions can have other locations, including the bones, lungs, subcutaneous connective tissue, trachea and more rarely the nervous system in domestic and wild avian species (Hawkey *et al.*, 1990; Randall and Reece, 1996; Beytut *et al.*, 2001; Zhu *et al.*, 2016) [2, 9, 17, 19].

The absence of macroscopic lesions on some viscera does not exclude the disease. In the same context, typical histological lesions of tuberculosis are mentioned on the lungs (Gonzalez *et al.*, 2002) [7] and the intestines (Kul *et al.*, 2005) [13] showing no lesion at the necropsy examination.

The frequency and severity of lesions in the intestines, suggesting oral infection. Spread of *Mycobacteria* from the intestine promotes the liver and the spleen contamination (Bowes, 1993) [3]. Finally, the germ is excreted mainly through the droppings. These contribute to the contamination of the rearing environment and eggshell at the time of laying and thereafter.

## 5. Conclusion

Avian tuberculosis was diagnosed in an adult hen issued from a backyard flock. The cachexia, the chronic diarrhea and the lesions observed at autopsy allowed us to suspect this disease. The recourse to the histopathological examination revealed necrotic and granulomatous lesions and the presence of acid-fast bacilli in various affected viscera. The existence of tuberculosis in backyard flocks is favored by the lack of biosecurity measures, the mixing of bird and animal species and the introduction in the farm of birds with unknown origin and sanitary status.

## 6. Conflict of interest

The authors declare that there is no conflict of interest.

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