

TUBERCULOSIS AND SARCOSPORIDIOSIS IN THE PERIORBITAL LOCATION IN A HEN

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Summary: In this report, avian tuberculosis, with visceral tuberculous lesions, and sarcosporidiosis in the periorbital location in a hen are described. A periorbital oedematous swelling was seen at the right eye of the hen. When the skin around the eye was peeled, a gelatinous effusion was detected in the subcutis. A few yellowish-white nodules of varying size were found in the visceral organs. Histopathologically, many early tubercles and only a few advanced tubercles were observed in the periorbital subcutis, in the same site sarcocysts were seen in the sarcoplasm of striated muscle cells. The lesions typical of tuberculosis were also detected in the visceral organs. Moderate numbers of acid-fast bacilli were visualised using Ziehl-Neelsen staining technique in paraffin sections. Löwenstein-Jensen medium with glycerin yielded *Mycobacterium* spp.

Key Words: Hen, tuberculosis, sarcosporidiosis.

Bir Tavukta Periorbital Bölgede Tüberkülozis ve Sarkosporidiozis

Özet: Sunulan vakada, bir tavukta visseral organlarda tüberküloz lezyonlarına ilaveten periorbital bölgede tüberkülozis ve sarkosporidiozis tanımlandı. Tavuğun sağ gözünde periorbital bölgede ödematöz bir şişkinlik görüldü. Göz çevresindeki deri yüzüldüğünde, subkutiste jel kıvamında sıvı efüzyonu saptandı. Visseral organlarda sarımsı-beyaz renkte az sayıda ve değişen büyüklüklerde nodüller gözlemlendi. Histopatolojik olarak, periorbital subkutiste çok sayıda genç ve az sayıda yaşlı tüberküller, çizgili kas hücrelerinin sarkoplazmasında sarkokistler ve visseral organlarda tipik yapılardan oluşan tüberküloz lezyonları tespit edildi. Parafin kesitlerin Ziehl-Neelsen ile boyanmasında az sayıda asido-rezistans basiller görüldü. Gliserinli Löwenstein-Jensen besiyerine yapılan ekimlerde *Mycobacterium* spp. üretildi.

Anahtar Sözcükler: Tavuk, tüberkülozis, sarkosporidiozis.

INTRODUCTION

The chronic and debilitating disease, avian tuberculosis (AT), caused by *Mycobacterium (M) avium*, affects the birds throughout the world^{1,2}. Although AT has been recognized as a separate contagious disease entity in birds for nearly a century, it continues to cause important losses in captive birds. A major problem in control of the disease is the ability of the organism to survive in the soil^{2,4}. However, commercial poultry farms are able to virtually eliminate the disease from their premises by slaughtering infected birds following diagnostic screening by intradermal tuberculin test, destroying contaminated equipment and housing, and isolating new stock from the contaminated environment^{2,4}.

Avian sarcosporidiosis is often found in individual birds. Some of the better known hosts are the chicken, domestic and wild mallard, black duck, and gadwall. Ducks are

especially likely to be affected, and the incidence of the disease is influenced by species, age, and geographic location of host⁵. However, the disease has rarely been reported in domestic chickens⁶⁻⁸.

In Kars city and its surrounds, avian tuberculosis is diagnosed sporadically in various avian species. Sarcosporidiosis has not previously been documented in any species of mammal or bird in the region. This case report describes avian tuberculosis with visceral tuberculous lesions and sarcosporidiosis characterized by periorbital oedematous swelling in the right eye of a hen in this region.

CASE REPORT

A 3 year-old hen from a small-scale family farm was submitted to the Department of Veterinary Pathology, University of Kafkas, for

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necropsy in September 2000. The hen appeared in poor physical condition and depressed. Its feathers were ruffled, and the soft feathers around the cloaca were matted with chronic diarrhoea. There was a periorbital oedematous swelling of about 3-4 cm in diameter at the right eye which, furthermore, could not be seen (Fig. 1). The hen was slaughtered and necropsied. Tissue samples were fixed in 10 % buffered formalin, processed routinely, and stained with hematoxylin and eosin (HE). Some sections were also stained using Ziehl-Neelsen (Z.N), Von Kossa and Congo red methods.

At necropsy, pectoral muscles were markedly atrophied, and the carcass was pale and emaciated. When the skin around the eye was peeled, a gelatinous yellowish effusion was detected in the subcutis. When the abdomen was opened, a few irregular yellowish-white nodules of size varying from 2-3 mm to 0.5 cm were found on the liver, spleen, and serosal surface of duodenum. A few caseonecrotic granulomas about 0.5-1 cm in diameter were observed in the lungs.

Histopathologically, the periorbital subcutis contained many early tubercles and, less commonly, advanced tubercles (Fig. 2). Early tubercles consisted of clusters of large, pale staining epithelioid cells. Advanced tubercles contained a central area of caseous necrosis, separated from the relatively normal tissue by a ring of inflammatory cells and thick fibrous capsule. The inflammatory reaction comprised predominantly of epithelioid cells, giant cells, heterophils, lymphocytes and plasmacytes. In addition, diffuse oedema, amyloid depositions, confirmed by positive staining with Congo red, and moderate numbers of heterophils were detected in the subcutis. In the same area, striated muscle cells (*m. orbicularis oculi*) revealed many sarcocysts in their sarcoplasms. The sarcocysts had double-layered walls and banana-shaped cystozoites. The interstitium around the muscle cells infected by sarcocysts was infiltrated by mononuclear cells (Fig. 3). No sarcocysts were found in the gluteal muscles.

The lesions typical of tuberculosis in the visceral organs were confirmed in the liver, spleen, gizzard, duodenum, cecum, and lungs. The histology of the tubercles was similar to described above. Amyloid depositions were found in the blood vessels, basement membranes, and around the periphery of advanced tubercles in the liver, spleen and gizzard. No calcium mineralization of tuberculous lesions was seen. Ziehl-Neelsen staining revealed moderate numbers of acid-fast bacilli which were free in the caseonecrotic debris and within the cytoplasm of epithelioid and giant cells.

Inoculations from the nodules in the liver and spleen were performed into the routine medium, Sabouraud Dextrose Agar (SDA), and Löwenstein-Jensen medium with and without glycerin. Inoculations into the routine medium and SDA yielded no specific agent. However, inoculations into Löwenstein-Jensen medium with glycerin yielded *Mycobacterium* species. Z.N staining performed on the colonies revealed acid-fast bacteria in clumps.

DISCUSSION

Although numerous publications on AT in various avian species are available^{1,2}, reports of the disease in chickens are very limited. Nonetheless, tuberculosis in chickens bred on small scale farms continues to cause unnecessary economic loss due to mortality, condemnation and diminished egg production, and remains a risk to man and susceptible farm animals^{1,2,4,9}. The disease is also diagnosed infrequently in turkeys, ducks (unpublished data) and geese¹⁰ in Kars city and its surrounds.

Even though tuberculous nodules may occur in any organ, lesions characteristic of the disease have rarely been reported in the subcutis, which is unusual site for localization of the disease^{9,11}. In this case, the presence of the lesions typical of avian tuberculosis characterized by oedema in the subcutis of the periorbital region was an unusual finding. Although avian tuberculosis in birds is generally caused by repeated ingestion of the organisms, it has been noted that the disease

may occur secondary to cutaneous lesions¹¹. Likewise, Pocknell et al.¹² reported conjunctival mycobacteriosis with visceral tuberculosis in two emus (*Dromaius novaehollandiae*), and the disease was attributed to such transmission routes. Thus, the entry of the organism through conjunctiva or skin abrasion was considered in this case.

Clinically, the hen revealed signs identical to those reported in chickens by other authors^{4,9}; diarrhoea, emaciation, and dullness. It has been documented that the localization of tuberculous nodules in birds occurs commonly in the liver and spleen, and less frequently in the lungs^{1,2,10}. Similarly, in this case, a few irregular yellowish-white nodules of size varying from 2-3 mm to 0.5 cm were found in the above mentioned visceral organs, and they were similar to those described in other avian species with tuberculosis^{10,11,13-15}. The presence of lesions typical of the disease in the alimentary canal indicated that the hen had shed organisms into the environment. The primary tuberculous involvement of the digestive tract in birds is believed to be the most important factor in spreading the causative organisms in the environment. Moreover, the bacilli have been reported to survive in soil and bird facilities for years^{1,2,14}. Organisms shed into the environment may be a potential pathogen for uninfected birds and other species in this region.

In both the subcutis and the visceral organs of the hen, early tubercles comprised of epithelioid cells and advanced tubercles contained a central area of necrosis surrounded by a pallisade of giant cells, and fibrous capsule around the periphery, consistent with the results of other authors^{9,11-13,15}. Amyloid depositions in this case were observed markedly in the visceral organs and the periorbital subcutis. Amyloidosis has been frequently associated with chronic infections, including tuberculosis, in various animal species and birds^{11,14}. In addition to its association with tuberculosis in birds, it has been noted as a primary disease in the waterfowls. However, whether amyloid deposition is immunologically mediated in the hen with tuberculosis was not evident in the present case.

It is known that tuberculosis in fowl is caused by *M. avium*^{2,3,13}. In this report, inoculations into Löwenstein-Jensen medium with glycerin yielded *Mycobacterium* species. However, facilities were not available for the performance of biochemical tests at the species level. Diagnosis was therefore based on the characteristic gross and histopathological findings, and on demonstration of acid-fast bacilli in the granulomas.

Although avian sarcosporidiosis is found throughout the world in individual birds, the disease has only infrequently been documented in domestic chickens^{5,6}. Sarcosporidiosis is known to be economically insignificant to the poultry industry, and does not appear to be a public health hazard. Nevertheless, mild signs have been reported by infected human volunteers⁵. In addition, sarcocystin, an endotoxin found in sarcocysts is reported to be toxic for rabbits, sparrows and mice and may slightly affect other animals^{5,6}. In birds as in mammals, sarcosporidiosis is recognized by the presence of elongated sarcocysts in the muscles^{5,8}. However, the lesions may be so small as to escape detection except by microscopic examination⁶. Similarly, in this case, sarcocysts were not seen in the skeletal muscles at necropsy, and were diagnosed histopathologically in the periorbital subcutis, but not in the gluteal muscles. However, it is not known whether sarcocysts were present in other skeletal muscles since histopathological examination was not performed. The microscopical features of sarcocysts in the striated muscle cells of the hen were in harmony with those reported by other authors^{5,8} who found that sarcocysts had double-layered walls, an inner spongy fibrous layer, and an outer dense limiting membrane. Although *Sarcocystis horwathi* is regarded as the etiologic agent for sarcosporidiosis in chickens⁵, etiological identification in this report could not be performed.

In conclusion, avian tuberculosis and sarcosporidiosis in the hen were diagnosed with reference to the gross and histopathological findings, and by demonstration of acid-fast bacilli. However, the sources of infection in this

case could not be determined. To date no correlation between avian tuberculosis and sarcosporidiosis has been established to our knowledge. The presence of avian tuberculosis and sarcosporidiosis in the same site, as reported here, may therefore be coincidental. Nonetheless, as a result of this finding, it may be worth conducting further detailed research into the potential effects of sarcosporidiosis in animal species.

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Figure 1. Periorbital oedematous swelling at the right eye of the hen.

Resim 1. Tavuğun sağ gözünde periorbital ödematöz şişkinlik.

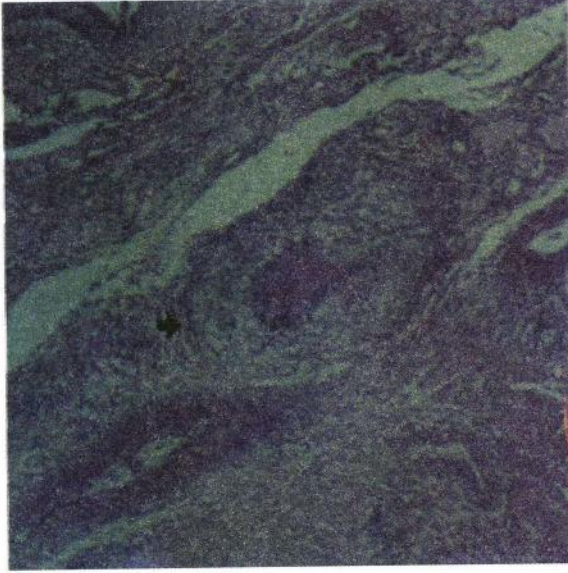


Figure 2. An advanced tubercle (arrow) containing caseation surrounded by giant cells and oedema in the periorbital subcutis. HE. Bar = 500 μ m.

Resim 2. Periorbital subkutiste ödem ve dev hücreleri ile çevrili kazeifikasyon nekrozu bulunan yaşlı tüberkül (ok). HE. Bar = 500 μ m.

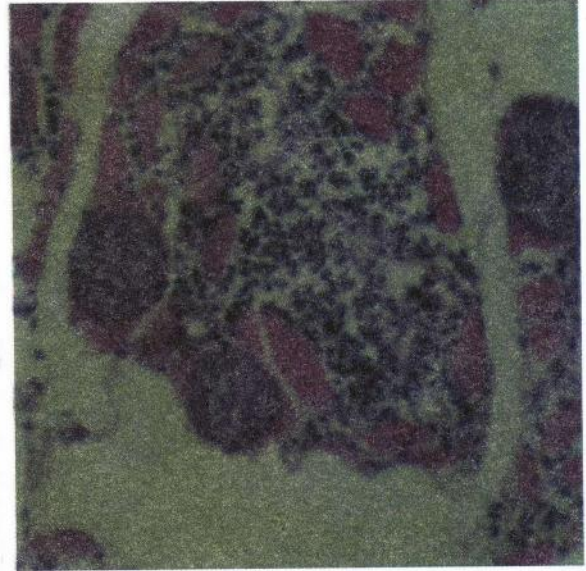


Figure 3. Sarcocysts in the sarcoplasm of the striated muscle cells and infiltrations of mononuclear cell in the periorbital subcutis. HE. Bar = 100 μ m.

Resim 3. Periorbital subkutiste çizgili kas hücrelerinin sarkoplazmasında sarkokistler ve mononükleer hücre infiltrasyonları. HE. Bar = 100 μ m.