

Generalized Amyloidosis in a Partridge (*Alectoris chukar*)

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Summary

This study describes a case of generalised amyloidosis in a rock partridge (*Alectoris chukar*). The case material was a 1 year-old male rock partridge submitted to the Department of Pathology, Faculty of Veterinary Medicine, University of Ankara for necropsy. In necropsy, the liver, spleen and kidneys were swollen, pale and fragile. Histopathologic examinations revealed pinkish amorph accumulation in spleen (on capsule, along reticular of white pulp, in the wall of blood vessel, white pulp, and trabeculae), liver (in the dysse spaces and on blood vessels' walls), kidneys (on tubule basal membranes and blood vessels' walls), proventriculus, gizzard (on lamina propria and blood vessels' walls), small intestines (on lamina propria, tunica muscularis, blood vessels' walls), pancreas (on blood vessels' walls), heart (on blood vessels' walls), lungs (on bronches and blood vessels' walls) and testes (on basal membranes of tubulus and blood vessels' walls). After staining Congo red, accumulations appeared in orange and in green under polarised light microscope. Immunohistochemically, amyloid substance stained positively against amyloid A protein. These accumulations were considered as amyloidosis, which is rare in exotic birds, but common in waterfowls.

Keywords: *Amyloidosis, Histopathology, Immunohistochemistry, Partridge*

Bir Keklikte (Kımalı Keklik) Generalize Amiloidozis

Özet

Bu çalışmada bir keklikte generalize amiloidozis olgusu tanımlandı. Çalışmanın materyalini Ankara Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na nekropsi amacıyla getirilen 1 yaşlı, erkek, kımalı keklik (*Alectoris chukar*) oluşturdu. Nekropside karaciğer, dalak ve böbrekler oldukça şişkindi. Solgun renkte olan bu organların kolayca parçalandığı dikkati çekti. Histopatolojik incelemede; dalakta (kapsülada, beyaz pulpanın ince retiküler ağı boyunca, beyaz pulpa ve trabeküllerdeki damar duvarlarında); karaciğerde (disse aralıklarında, ve damar duvarlarında); böbreklerde (tubulusların bazal membranında ve damar duvarlarında); duodenum ve jejenumda (lamina propria'da, damar duvarlarında, tunika muskularis'te); kaslı ve bezli midede (lamina propria ve damar duvarlarında); pankreasta (damar duvarlarında); kalpte (miyofibrillerde); akciğerlerde (parabronşlarda ve damar duvarlarında); testislerde (tubulus bazal membranında ve damar duvarında) homojen, amorf pembe renkte birikimler gözlemlendi. Yapılan Congo-red özel boyasıyla bu birikimlerin kırmızimsı portakal renginde boyandığı, polarize ışık mikroskobu altında ise yeşil yansıma verdiği gözlemlendi. Yapılan immunohistokimyasal incelemede amiloid maddesi amiloid A(AA) proteinine karşı pozitif reaksiyon verdi. Bu veriler ışığında anılan birikimlerin kanatlı hayvanlar arasında özellikle su kuşlarında rastlanan, ancak diğer kanatlılarda nadir görülen amiloid birikimi (AA amiloid) olduğu saptandı.

Anahtar sözcükler: *Amiloidozis, Histopatoloji, Immunohistokimya, Keklik*

INTRODUCTION

This study presents a case of generalized amyloidosis encountered in a partridge. Amyloidosis is defined as the extracellular accumulation of nonsoluble autologous or precursor proteins, in fibrillary form, in various tissues and organs, and resulting dysfunction of these organs

caused by the loss of parenchymal cells and micro-anatomical structure. Generalized amyloidosis, except for water fowl, is reported to be encountered rarely in avian species¹⁻³ yet, the incidence is indicated to be high particularly in water fowl pertaining to the orders



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Anseriformes, *Gruiformes* and *Phoenicopteriformes* ^{2,3}. Amyloidosis, among species belonging to the order *Anseriformes*, occurs more frequently in the *Anatidae* (ducks, geese, swans) ^{1,3-5}. Furthermore, in these avian species, amyloid deposition is the type AA (Amyloid Inflammation Associated), which is accompanied by chronic inflammatory disorders ^{2,3,6}.

CASE HISTORY

The case material was a 1 year-old male rock partridge submitted to the Department of Pathology, Faculty of Veterinary Medicine, University of Ankara for necropsy.

Histopathology: Systemic necropsy was performed on the partridge and the tissue samples taken at necropsy were subjected to the routine tissue processings. The 5-micron thick cross sections cut from the paraffin blocks

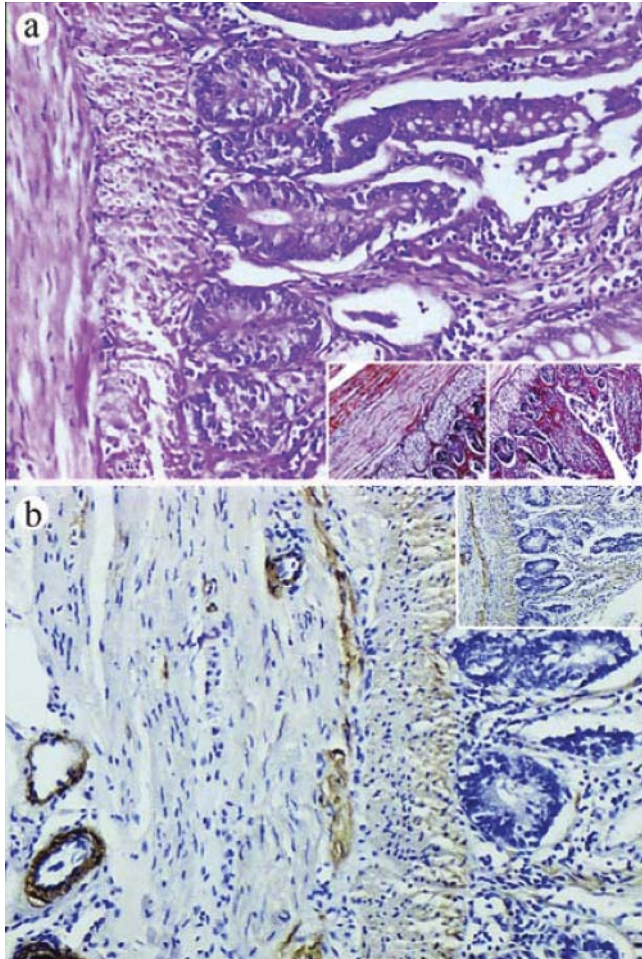


Fig 1. a - Amyloid deposits around blood vessels, lamina propria and tunica muscularis of the small intestine . HE x 40, small plates Congo red stain x 40, **b** - Amyloid positively stained with mc1 in small intestine. Immunohistochemical staining x 40

Şekil 1. a - İnce bağırsaklarda kan damarlarının çevresinde, tunika muskularis ve lamina propriada amiyloid birikimi. HE x 40, küçük resim Congo red x 40, **b** - mc1 ile ince bağırsaklarda amiloid pozitif. Immunohistokimyasal boyama x 40

were stained with hematoxylin-eosin (HE). Selected cross sections were stained specifically with Congo-red and Alcian blue ⁷. The preparations were examined under light microscope and polarized light microscope.

Immunohistochemistry: Paraffin cross sections were stained with the streptavidin-biotin-peroxidase staining technique. For peroxidase staining, amyloid-A mouse monoclonal antibody (Clone mc1; Dako) was used at a dilution of 1:250. Furthermore, for localisation of IgG, rabbit polyclonal antibodies against chicken IgG (H+L; Nordic Immunological Laboratories, Tilburg, The Netherlands) were used at a dilution of 1:100. 3-amino-9-ethylcarbazole (AEC substrate-chromogen, DAKO, Denmark) served as a chromogen ⁸.

Macroscopic examination revealed the liver, spleen and kidneys to be rather swollen. These organs, which were determined to be of pale colour, were also friable. Furthermore, heavy parasitic infection was observed in the small intestines.

Histopathological examination revealed the presence of homogenous, pink-coloured amorphous deposits, which were widespread in the capsule and white pulp of the spleen, Dysse's spaces of the liver, lamina propria and tunica muscularis of the duodenum and jejunum (*Fig. 1a*), in-between the glands in the lamina propria of the muscular and glandular stomach, the basal membrane of the tubuli of the testes, and the basal membrane of the tubuli and the glomeruli of the kidneys, and which were found a lesser extent in the parabronchi of the lungs. Furthermore, similar deposits were also observed in all of the listed organs blood vessel's walls as well as

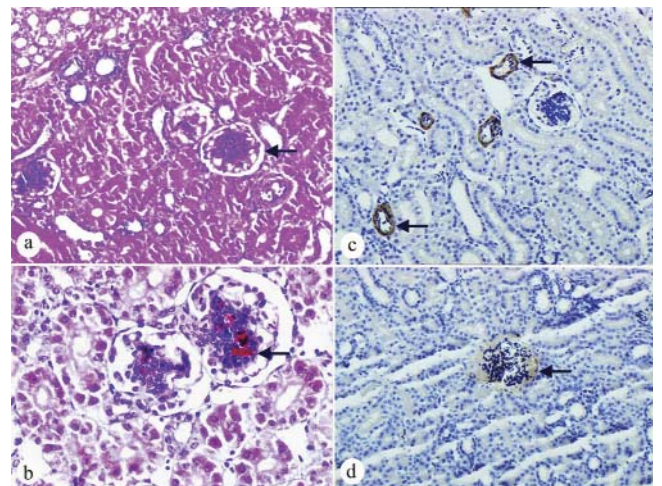


Fig 2. a - Amyloid deposits in the glomeruli Congo red stain x 10 (arrows), **b-c** - Immunohistochemical staining of amyloid deposits in kidney. Amyloid positively stained with mouse monoclonal antibody mc1 with x 10 (arrows)

Şekil 2. a - Glomerulusta amiloid birikimi. Congo red x 10 (oklar), **b-c** - mc1 ile böbrekte amiloid birikimi pozitif. Immunohistokimyasal boyama x 10 (oklar)

in the pancreas (Fig. 3a) and the walls of blood vessels in the heart. When stained with Congo red, these deposits were recognized by their reddish orange colour (Fig. 1a, 2a, 3a) and they were determined to show green birefringence when viewed under the polarized light microscope. When Alcian blue was applied, the amyloid deposits found in the aforementioned locations, which were observed to stain blue (Fig. 4a-b).

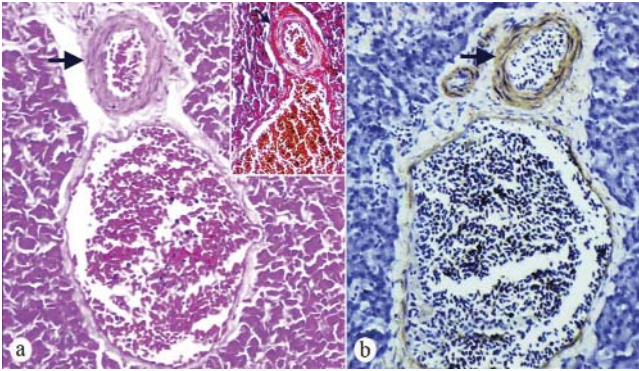


Fig 3. a - Amyloid deposits around blood vessels in pancreas HE x 40 (arrow). Above small plate Congo red stain x 40 (arrow), **b** - Immuno-histochemical staining of amyloid deposits in pancreas. Amyloid positively stained with mouse monoclonal antibody mc1 with x 40 (arrow)

Şekil 3. a - Pankreasta kan damarlarının çevresinde amiloid birikimi HE x 40 (ok), Küçük resim Congo red x 40 (ok), **b** - mc1 pankreasta amiloid birikimi immunohistokimyasal boyama x 40 (ok)

By means of immunohistochemistry, the biochemical typing of amyloid was able to be made, and the amyloid determined to have accumulated in the listed organs was determined to give positive reaction with amyloid A (AA) protein (Fig. 1b, 2b-c, 3b).

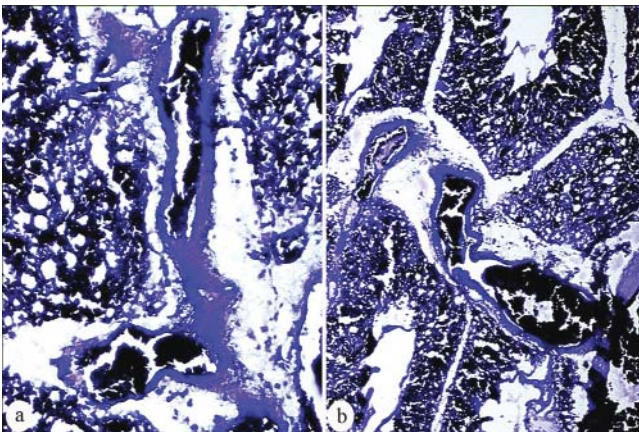


Fig 4. a-b - Amyloid deposits in the parabronchi of the lungs. Alcian blue, x 40

Şekil 4. a-b - Akciğerlerde parabronşlarda amiloid birikimi. Alcian blue, x 40

DISCUSSION

Systemic AA amyloidosis does not display any specific clinical symptom in either wild birds or domestic poultry.

At macroscopic level, the spleen, liver, kidneys and small intestines are affected most frequently^{1-3,9}. However, the proventriculus, gizzard, heart, large intestines^{3,8-10} endocrine organs and gonads are less affected, whereas the brain, lungs and skin are affected rarely^{1,2,5,8,9}. Macroscopically, the liver and spleen enlarge and display subcapsular haemorrhages or conversion to a bronze colour. The kidneys are pale and swollen^{1,3,5,9-11}. In some instances, the heart displays dilatation of the left ventricle, hydro-pericardium or ascites¹⁰. Microscopic examination reveals the presence of amyloid deposits in the Disse's spaces, Glisson capsule and blood vessel walls of the liver, the capsula and white pulp^{5,9-11} as well as the blood vessel walls^{1,5,8-11} and red pulp^{1,8} of the spleen, the interstitium, basal membrane of the tubuli and blood vessel walls of the kidneys⁸⁻¹⁰ and sometimes the adventitial layer of the ureters¹⁰. Amyloid deposits are also observed in glomeruli, although to a less extent^{1,8}. Amyloid accumulation has also been determined in the duodenum and jejunum in the lamina propria and wall of blood vessels⁸⁻¹¹. In cases of severe amyloidosis, the presence of amyloid has also been determined in the lamina propria of the proventriculus as well as in the interstitium of proventricular glands^{1,9-11}, and the wall of blood vessels in the heart and pancreas. However, amyloid accumulation has not been determined in the myocardium^{9,10}. Staining with Congo red produces a colour ranging from orange to red in amyloid deposits, and is determined to show birefringence under the polarized light microscope^{10,11}. Furthermore, amyloid is ascertained to stain blue with Alcian blue¹⁰.

The macroscopic and microscopic lesions observed in the present case study were determined to be similar to those reported in the literature, yet, amyloid deposits were not observed in the red pulp of the spleen in this case study. However, amyloid accumulation was determined to be intense in the muscular and glandular stomach, testes and pancreas, which are organs reported to be rarely affected by amyloidosis, and less intense in the lungs and heart.

It is possible to determine the chemical type of amyloid by means of immunohistochemical techniques in which specific antisera against amyloid proteins are used¹². The type of amyloid most frequently encountered in animals is reactive amyloid, namely AA amyloid, of which the amyloid fibrils are formed by amyloid protein A^{1,6,8,10,12}. AA amyloidosis develops as a result of long lasting chronic infections (tuberculosis, tumours, viral or bacterial diseases) or predisposing factors such as stress. Such factors are reported to increase the level of serum amyloid A (SAA) and to cause it to breakdown and precipitate in tissues, thereby resulting in the formation of specific amyloid fibrils¹³. Ducks constitute a significant model for amyloidosis research in avian

species⁵. Similar studies have also been conducted in other avian species, in which tumours^{3,4} and chronic infections caused by bacteria^{1,3,8,9,11,14-16} or parasites³ and viruses^{3,17} as well as those associated with vaccination³ and stress^{5,3} resulted in AA amyloidosis. Immunohistochemical staining performed in the present study showed the type of amyloid, accumulated in the listed organs of the partridge examined, to be secondary amyloid or AA type amyloid. Furthermore, the determination of a severe infection of *Ascaridia numidae* in the small intestines suggested the partridge to have developed generalized amyloidosis probably due to chronic parasitic infection.

In conclusion, only a few cases of amyloidosis have been reported in avian species belonging to the order Galliformes^{8,10,14,15}. Except for the report of amyloid accumulation in the sinuses of a partridge infected with *M. gallisepticum* by McMartin et al.¹⁴, systemic amyloidosis has not been reported previously in this avian species. The present case study provides the histopathological and immunohistochemical definition of systemic amyloidosis, which is encountered rarely in the partridge.

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