



NOTE

Avian Pathology

Systemic amyloid A (AA) amyloidosis in the Bengalese finch (*Lonchura striata var. domestica*)

Yumiko NAKANO¹⁾ and Hiroo MADARAME^{2)*}¹⁾Nakano Bird Clinic, 3-35-10 Wakamiya, Nakano-ku, Tokyo 165-0033, Japan²⁾Veterinary Teaching Hospital, Azabu University, 1-17-71 Fuchinobe, Chuo, Sagami-hara, Kanagawa 252-5201 Japan*J. Vet. Med. Sci.*

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ABSTRACT. The incidence of systemic amyloid A (AA) amyloidosis was high in Bengalese finches (*Lonchura striata var. domestica*), as 150 of 235 birds from 5 breeding colonies (63.8%) tested positive for it. The incidence was statistically significantly higher in one colony. The liver was the most frequently affected organ (150 cases), followed by the spleen and then the kidneys. The degree of amyloid deposition was high in the liver and spleen and low in the kidneys. The histological distribution of amyloid deposits was similar in all finch species. Species-specific or colony-specific predisposing factors for systemic AA amyloidosis in Bengalese finches remain to be elucidated. As far as we know, this is the first report of amyloidosis in Bengalese finches.

KEY WORDS: amyloid A (AA), Bengalese finch, systemic amyloidosis

Amyloidosis is a group of diseases and is characterized by the extracellular deposition of amyloid protein within various organs in humans and animals [13]. Amyloidosis can be classified into localized or systemic types, depending on the pattern of amyloid deposition [1].

Amyloidosis is a well-recognized pathological disorder in birds. A majority of spontaneous amyloid cases have been characterized by amyloid A (AA) deposition in virtually all tissues except the brain parenchyma [6, 13]. Amyloid- β protein deposition has also been described in the brain of birds [4, 7, 11]. Although the true incidence of avian amyloidosis is difficult to assess, the highest recorded incidence in birds is in Anseriforms, especially in the Anatidae family (e.g., swans, geese, and ducks) [3, 5, 6]. In contrast, amyloidosis is a relatively rare disease in other birds, including doves, finches, and parrots [12].

Amyloidosis was reported in three finch species of the order Passeriformes: the Gouldian finch (*Erythrura gouldiae*) [18], the Australian diamond firetail finch (*Stagonopleura bella*) [2], and the zebra finch (*Taeniopygia guttata*) [9, 13, 14]. A high incidence of AA amyloidosis was observed in a colony of zebra finches recently, but the actual incidence rate has not been determined [13, 14].

The Bengalese finch (*Lonchura striata var. domestica*) species was created artificially in China or Japan, with roots in the white-rumped munia (*Lonchura striata*) [16]. The Bengalese finch is easy to care for and can be easily bred [16]. It is one of the best known and most common pet among the order Passeriformes, in addition to being a laboratory animal in Japan [10].

Bengalese finches with abdominal distension were occasionally found to have died in some breeding colonies over the last several years, and amyloidosis was diagnosed in these birds postmortem. Therefore, a retrospective necropsy survey was conducted to determine the incidence of amyloidosis in dead Bengalese finches in breeding colonies. A histological investigation focused on the three visceral organs that are most frequently affected by avian amyloidosis, i.e., the liver, spleen, and kidneys [5, 6, 13, 14].

Two hundred and thirty-five dead Bengalese finches from 5 breeding colonies were collected and analyzed between 2006 and 2019. The birds were found to have died without any clinical description. Breeding colonies A and B were inbred and parent birds were introduced into colony B from colony A. Breeding colonies C, D, and E were outbred and parent birds were purchased from different vendors. The liver, spleen, and kidneys were immersed in 10% neutral buffered formalin solution for the necropsy survey. Paraffin sections were prepared according to the standard method and stained with hematoxylin and eosin (HE), and Congo red. The Congo-red stained sections were observed under polarized light. Immunohistochemistry was performed to detect AA protein using an anti-AA antibody (MX-Amyloid A, mouse monoclonal antibody, Kyowa Medix) and Histofine Simple Stain MAX-PO (MULTI) kit (Nichirei, Tokyo, Japan).

The degree of amyloid deposition within each organ was divided into three grades according to the following criteria: “mild” amyloid deposition, which replaced little or none of the parenchyma; “severe” amyloid deposition, which extensively replaced

*Correspondence to: Madarame, H.: madarame@azabu-u.ac.jp

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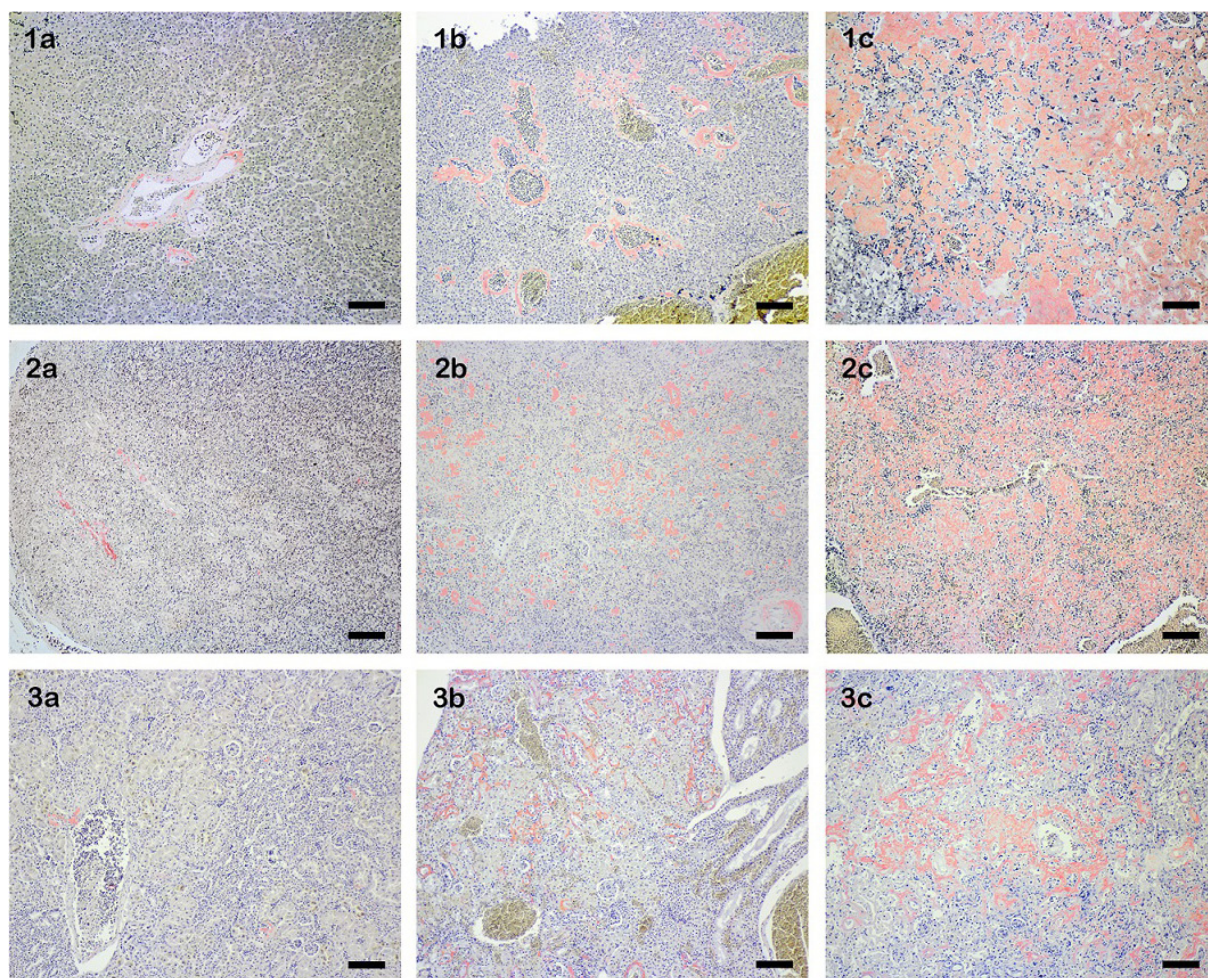


Fig. 1. Grading system of amyloid deposition within the liver (1), spleen (2), and kidneys (3). The degree of amyloid deposition was classified based on histological findings, mild (a), moderate (b), or severe (c). Congo red. Bar=100 μ m.

Table 1. Amyloid deposition grading score of the liver, spleen, and kidneys

Grade	Liver	Spleen	Kidneys
Severe	89	90	4
Moderate	33	27	21
Mild	28	24	85
Total number	150	141	110

Table 2. Age composition in Bengalese finches affected systemic amyloid A amyloidosis

Age	2 years ^{a)} <	2–4 years	4–6 years	6–8 years	8 years <	Total
Amyloid (+)	11	37	34	10	2	94
Amyloid (–)	16	10	8	1	0	35
Total	27	47	42	11	2	129

a) The incidence of affected birds was significantly lower than that of other age groups. $P < 0.05$, $\chi^2 = 18.85$, $df = 4$.

a considerable portion of the parenchyma; and “moderate” amyloid deposition, which was an intermediate between the mild and severe grades (Fig. 1 and Table 1).

The effect of aging on amyloidosis was analyzed by dividing every 2 years of age (Table 2). In general, Bengalese finches more than 4 months of age are considered adult birds [19]. The life span of a Bengalese finch is between 4 and 7 years and maximum life span is 7 years [15, 17].

The χ^2 test was used to compare the sex- and age-based incidences of amyloidosis. Residual analysis was used to determine which age groups of birds are significantly different in amyloidosis incidence. The Fischer’s exact test was used to compare differences in incidence between colonies. Ryan’s multiple range tests were used to compare the amyloidosis incidences of pairs of each colony. P -values < 0.05 were considered statistically significant.

Amyloid deposits appear acidophilic and homogeneous on HE staining and red-orange on Congo-red staining, and exhibit apple-green polarization under polarized light (Fig. 2). The intensity of Congo-red staining and immunostaining for the anti-AA antibody were consistent (Fig. 2).

Amyloid deposition was observed in the liver of 150 birds, followed by the spleen (141 birds), and kidneys (110 birds). The degree of amyloid deposition was high in the liver and spleen, and low in the kidneys (Table 1). Histologically, amyloid deposition in the liver was observed mainly in the space of Disse and along the basement membrane and walls of the central and interlobular veins. Amyloid compressed the hepatocytes and replaced the hepatic parenchyma in severe cases. Amyloid deposition was observed along the basement membrane of the sheathed arteries in the spleen. Amyloid filled the sinuses and effaced the splenic parenchyma in severe cases. Amyloid deposition occurred mainly along the basement membrane of the renal tubules, along the basement membrane and wall of the intralobular vein in the kidneys. Amyloid deposits extended into the renal interstitium in severe cases.

The incidence of systemic AA amyloidosis was 150 in 235 dead Bengalese finches (63.8%) (Table 3). The chi-square test showed a significant age-based difference in the incidence of amyloidosis, but no significant sex-based difference (Table 4). Residual analysis showed that the incidence of affected birds less than 2 years of age was significantly lower than that of the other age groups (Table 2). Fischer's exact test showed that the incidences of amyloidosis was significantly different between the five colonies as a whole bird population. Ryan's multiple range tests showed the incidence of amyloidosis in colony A differed significantly from that in the other four colonies (B, C, D, and E; Table 3).

The frequency of organ involvement in avian amyloidosis varies among species [12]. In the finch species, amyloid deposition is more common in the liver and spleen [12]. In zebra finch, the degree of amyloid deposition was remarkable in the liver and spleen [9] and the liver was the most frequently and severely affected organ [13]. The histological distribution of amyloid deposition is similar to each other in finch species [9, 12–14].

The highest recorded incidence of avian amyloidosis was in Anseriforms, especially in the Anatidae family. The incidence of avian amyloidosis in waterfowls from two zoological gardens was 20% and 45%, respectively [5], and ranged from 5% to 40% in Pekin ducks [12]. Amyloidosis is relatively rare in other birds, including doves, finches, and parrots [12]. Among pet birds, amyloidosis is more common in the small passerines species [12]; the recorded incidence of amyloidosis in a five-year retrospective review was 3% in Passeriformes [8].

Five case studies investigated amyloidosis in three finch species (from the order Passeriformes): one study included 4 Gouldian finches from a zoo [18], one study included an Australian diamond firetail finch from a pet store [2], another study included a companion zebra finch with amyloidosis [9], and two studies included a total of 14 zebra finches with AA amyloidosis from a research breeding colony [13, 14]. Recently, a high incidence of AA amyloidosis was observed in zebra finches,

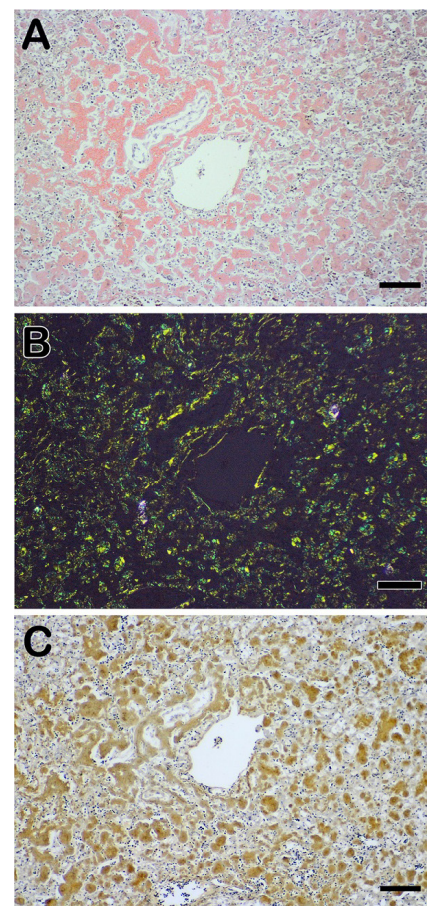


Fig. 2. Severe amyloid deposition at the same area in the liver. The homogeneous deposits stained with Congo red (A), showed apple-green polarization under polarized light (B), and the deposits were positive for the anti-amyloid A antibody (C). Bar=100 μ m.

Table 3. Incidence of amyloid deposition in the Bengalese finch

	Total No. of birds	No. of birds affected amyloidosis	Males	Females	Sex unknown	Incidence (%)
Colony A	100	81	42	39	0	81 (81/100)
Colony B ^{a)}	26	15	7	8	0	57.7 (15/26)
Colony C ^{a)}	65	31	13	18	0	48.5 (31/65)
Colony D ^{a)}	40	23	12	10	1	57.5 (23/40)
Colony E ^{a)}	4	0	0	0	0	0 (0/4)
Total	235	150	74	75	1	63.8 (150/235)

a) The incidence of amyloidosis in colony A differed significantly from that in the other 4 colonies. $P < 0.05$.

Table 4. Incidence of amyloid deposition

	Males	Females	Total
Amyloid (+)	74	75	149
Amyloid (-)	35	46	81
Total	109	121	230

Sex difference was not statistically significant. $P < 0.05$, $\chi^2 = 0.88$, $df = 1$.

but the actual incidence rate in colonies has not been determined [13, 14].

The development of systemic AA amyloidosis in birds has been associated with aging, breed, chronic inflammation, trauma, various infectious and neoplastic diseases, stress linked to overcrowding, and possible genetic predispositions [8, 9, 12, 13]. Amyloidosis (in finch species) was accompanied by mycobacteriosis in 4 Gouldian finches [18], proventricular cryptosporidiosis in an Australian diamond firetail finch [2], and testicular interstitial tumor and enteric salmonellosis in a zebra finch [9], and 5 birds from a captive zebra finch colony showed clinical features of various inflammatory, infectious, and neoplastic conditions [13]. It is unknown whether zebra finches have a higher genetic susceptibility to AA amyloidosis or if it was the case only in certain colonies [13]. The causes of systemic AA amyloidosis have not been conclusively identified [2, 9, 13, 18].

In the present study, systemic AA amyloidosis in the Bengalese finch was associated with age but not sex. There was no significant difference between the incidence of amyloidosis and preexisting inflammatory or neoplastic conditions (unpublished data). Effects of inbreeding are postulated for colony-specific genetic susceptibility to AA amyloidosis, although other predisposing factors are not conclusively ruled out.

In conclusion, a high incidence of systemic AA amyloidosis was observed in Bengalese finch breeding colonies and the incidence was statistically significantly higher in one colony. This is the first report of systemic AA amyloidosis in the Bengalese finch. The etiology of AA amyloidosis has not been identified in the Bengalese finch. Species-specific or colony-specific predisposing factors for systemic AA amyloidosis also remain to be elucidated.

CONFLICT OF INTEREST. The authors declare no conflict of interest with respect to the publication of this manuscript.

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