Systemic AL amyloidosis in a Beech Marten (*Martes foina*)

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ABSTRACT

A wild Beech Marten (*Martes foina*), was referred for necropsy to the Department of Animal Pathology of the University of Turin (Italy).

At gross examination, whitish and firm masses, 10-mm in diameter, were found on the heart and in the kidney. Spleen showed lighter color and greater consistency, and the cut surface of the liver appeared scattered with whitish-yellow coalescing foci homogeneously distributed.

Amyloid deposits were present in the perivascular and intercellular spaces of the visceral organs, such as the heart, liver, and kidneys. Amyloid stained positively with Congo red with and without 5% potassium permanganate pretreatment and showed green birefringence observable under polarized light. A diagnosis of systemic AL amyloidosis was made. This is the first description of systemic AL amyloidosis in a wild Stone Marten.

Amyloidosis is not a single disease but a term for diseases that share a common feature: the extracellular deposition of pathologic insoluble fibrillar proteins in organs and tissues that shows the following characteristics: beta-pleated sheet molecular configuration (Cohen and Calkins, 1959; Shirahama and Cohen, 1967; Bonar et al., 1969) with affinity to Congo red dye, that appears red microscopically in normal light but apple green when viewed in polarized light (Bennhold, 1922; Divry and Florkin, 1927), fibrillar ultrastructure and localization in the extracellular matrix, that leads to hardening of the affected tissues. Depending upon its extent, amyloidosis can be classified as a systemic or localized disease.

Two major forms of systemic amyloidosis have been described. The most common form in domestic animals is reactive (secondary) amyloidosis due to chronic inflammatory diseases. In reactive amyloidosis, the deposited amyloid protein is AA type and it derives from serum amyloid A, which is synthesized in the liver. The other form of systemic amyloidosis is light-chain (AL) amyloidosis, which is the most common type in humans but is very rare in domestic animals (Kim et al., 2005).

A wild female Beech Marten (*Martes foina*), about one year old, found in a garden in Ventimiglia (Italy), was referred for necropsy at the Department of Animal Pathology of the University of Turin (Italy) because it died 24 h after receiving symptomatic therapy.

The animal was in very poor condition, it presented respiratory difficulties and depression.

Tissue samples from the lung, liver, spleen, heart, brain and kidney were fixed in 10% neutral buffered formalin (pH 7) and paraffin-embedded for histological examination. Four-μm sections were cut using a microtome, stained with haematoxylin and eosin and Congo red with and without pretreatment of 5% potassium permanganate solution, and examined by light microscopy. Sections of dog AA amyloidosis and human AL amyloidosis were run concurrently as control.

Samples of spleen and liver processed for ultrastructural evaluations were fixed in 2.5% gluteraldehyde phosphate (pH 7.3) and stored at 4 °C for 24 h. After the post-fixation process (in 1% osmium for 2 h and a quick wash out in 30% acetone) the samples were dehydrated in acetone, and Spurr resin embedded. From each sample, using the ultramicrotome, thin sections (0.90 μm) stained with toluidine blue were obtained, and afterwards ultrathin sections of 70 nm contrasted by uranyl acetate and Pb citrate. The grids were evaluated using a transmission electron microscope (CM10 Philips, Koninklijke Philips Electronics N.V. 5621 BA, Eindhoven, the Netherlands). Bone marrow, liver and spleen were collected for bacteriological tests which were performed on blood–agar. Moreover, a direct immunofluorescence test for rabies virus was applied on impression smears from nervous tissues.

At necropsy the myocardium was pale and in the ventricular walls there were some well defined whitish areas (0.2–0.4 cm × 0.5^{-1} cm). Localized at the apex there was a white-greyish fibrous area (2 cm) protruding from the surface. At cross

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section the lesion appeared to extend throughout the wall of the left ventricle and part of the right one. In the cranial pole of the right kidney a large white-yellowish fibrous lesion was present. Spleen showed increased volume and rounded edges. The liver was enlarged with irregular white-greyish areas slightly protruding. The cut surface appeared scattered of whitish-yellow coalescing foci homogeneously distributed.

Histologically, under the capsule, in the walls of the blood vessels and in the portal areas of the liver there was an accumulation of eosinophilic homogeneous and amorphous material (Fig. 1a). The hepatocytes showed diffuse degeneration with homogeneous and often vacuolised cytoplasm. The glomerular tufts of the kidneys were enlarged and the basement membrane of the capillaries was infiltrated by eosinophilic amyloid. The lumen of the glomerular vessels was obliterated and degenerative alterations were evident in the epithelium of the tubules (Fig. 1b).

In the heart a deposition of homogeneous matter within the walls of the arterial and venous vessels was found. In proximity of the apex of the left ventricle there was a large area of necrotic tissue, surrounded by a thick connective capsule; the arterial vessels around the lesion had a subendothelial deposition of homogeneous material which in some cases was eccentric while in some other involved the entire vessel wall.

With the exception of the vascular congophilic material, lungs were not affected, and amyloid could not be detected in the nervous tissue.
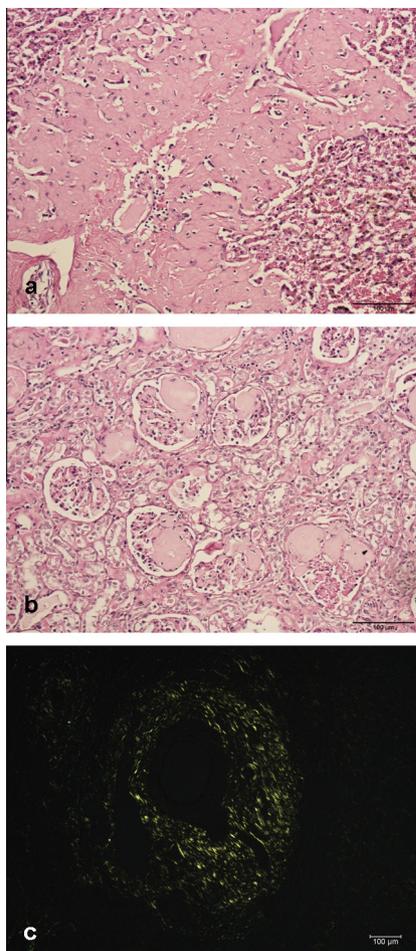
Congo red staining with polarization revealed apple green birefringence characteristic of amyloid. Congophilic material was also observed with Congo red staining pretreated with potassium permanganate allowing the diagnosis of AL amyloidosis (Fig. 1c) and transmission electron microscopy was used to confirm the presence of amyloid fibrils: spleen and liver cells were surrounded by masses of amyloid fibrils in cross and longitudinal sections measuring approximately 8 nm in diameter, randomly oriented, localized in the extracellular space, forming a dense network of fibers (Fig. 2).

The bacteriological and the direct immunofluorescence tests for rabies virus were negative.

The most frequently encountered amyloid type in veterinary medicine is AA-amyloid due to chronic inflammatory diseases (Gruys, 2004; Kim et al., 2005) and it has been previously reported in Stone Marten (Wandeler and Pauli, 1969; Linke et al., 1980). The other forms of systemic amyloidosis are light-chain (AL) amyloidosis, familial amyloidosis, senile systemic amyloidosis, and hemodialysis-associated amyloidosis (Falk et al., 1997).

Local deposition of AL-amyloid is reported in various species of animals. This includes diffuse to nodular, tracheal, and bronchial AL-amyloidosis in dogs (Labelle et al., 2004; Besancon et al., 2004) and cutaneous nodular amyloidosis in equines (Linke et al., 1991; Woldemeskel, 2012). Systemic AL amyloidosis has only been reported in horses (Hawthorne et al., 1990; Kim et al., 2005), in a cat (Carothers et al., 1989), and in a cow, associated with bovine leukocyte adhesion deficiency (Taniyama et al., 2000).

Resistance to permanganate oxidation is a procedure that reduces the affinity of amyloid protein AA for Congo red (Wright et al., 1977; Van Rijswijk et al., 1979). This feature is useful in the preliminary differentiation of reactive from other types of amyloidosis because resistance to permanganate oxidation suggests that the amyloid deposits in this Stone Marten did not contain amyloid protein AA.

In human pathology, histologic examination of systemic AL amyloidosis reveals some degree of amyloid deposition in virtually every organ system except the central nervous system (Falk et al., 1997) such as in this case.

In human clinical practice, amyloidosis is classified as primary, secondary, hereditary, and age related (Falk et al., 1997; Kholovà and Niessen, 2005). Primary (idiopathic, systemic) amyloidosis appears with no antecedent or coexisting disease, it involves

Fig. 1. *Martes foina*: eosinophilic homogeneous and amorphous material into the walls of the blood vessels of the liver (a) (H&E), of the kidney and in the glomerular vessels (b) (H&E). (c) Homogeneous and amorphous material positive for Congo red stain with pretreatment of 5% potassium permanganate solution in a vessel of the kidney (polarized light).
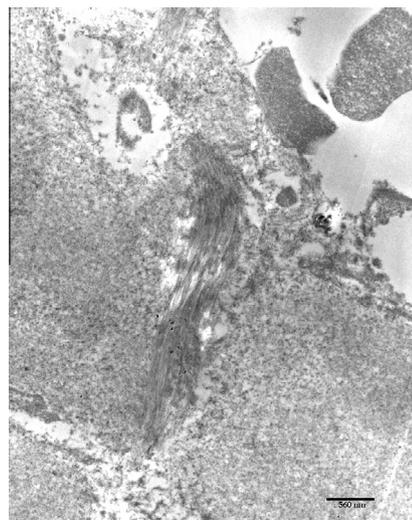

Fig. 2. *Martes foina*, spleen: transmission electron microscopy photomicrograph of amyloid fibrils in cross and longitudinal sections, randomly oriented, extracellular, forming a dense network of fibers.

mesenchymal organs such as the cardiovascular system, gastrointestinal tract, and muscle tissue, and tends to form nodular deposits. Cardiac involvement is common (Kyle and Bayrd, 1975; Pascali, 1995; Falk et al., 1997; Kholovà and Niessen, 2005). Cardiac deposition of amyloid was not observed in the other reports of AL systemic amyloidosis in animals contrary to our case where both grossly and histologically deposits of AL amyloid in the heart were present.

Hepatic amyloidosis occurs in most species of domestic animals and amyloid can accumulate in more than one pattern. It may accumulate in vessel walls and within the connective tissue of the portal area, and in the space of Disse, where it impairs the normal access of plasma to hepatocytes. Even though extensive hepatic damage occurs and many hepatic cells are destroyed, death may be caused by rupture and massive haemorrhage into the peritoneal cavity (Mcgavin and Zachary, 2006).

Deposition of amyloid in the kidneys is a relatively common phenomenon in systemic amyloidosis. The amyloid deposits in the kidney are found in the glomerular tuft and around the capillaries in the interstitial tissue, between the straight tubules. Most of the renal damage is due to obliteration of the glomerular circulation and deposition of amyloid between the capillary endothelium and the epithelium of the glomerular tuft with consequent stenosis and obliteration (Mcgavin and Zachary, 2006).

Because it leads to progressive renal impairment and eventual chronic renal failure, renal amyloidosis poses one of the major clinical considerations of systemic amyloidosis (Looi and Cheah, 1997).

Although amyloidosis is reported in different domestic animals and among wild species with a particularly frequency in mustelids, our case is characterized by systemic deposition of type AL amyloid (an event rarely described in veterinary pathology) with special involvement of kidney, liver, heart and spleen.

Histochemical characterization of lesions revealed a type AL amyloidosis by resistance with pretreatment with potassium permanganate at Congo red staining, a quick and effective method for distinguishing the main forms of amyloidois. TEM further supported the diagnosis.

Conflict of interest

The authors disclose any financial and personal relationships with other people or organisations that could inappropriately influence this work.

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