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Case Report

A case-report of biochemistry and serum amyloid A in a moribund free-ranging Baltic herring gull (*Larus argentatus*) with necrotic wing fracture

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Abstract

An adult herring gull (*Larus argentatus*) found lethargic and moribund showed an open fracture of the right radius and ulna with necrosis of the surrounding tissue. Hematologic testing and plasma biochemical analysis revealed elevated creatinine kinase consistent with traumatic muscle damage in addition to hyperuricemia, hyperkalemia, and hyperphosphatemia consistent with renal insufficiency. An increase in the acute phase protein serum amyloid A (SAA) indicates a high degree of inflammation supported by leucocytosis, heterophilia, and hypoglycemia pointing towards septicemia. This case provides knowledge about Serum Amyloid A in gulls and how bone fracture and secondary infection may affect gull blood hematology and biochemistry.

Keywords: Baltic, Herring Gull, Sepsis, Serum Amyloid A (SAA), Heterophilia

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The Case

One adult herring gull (*Larus argentatus*) of unknown sex and age was found in 2018 lethargic and moribund at Christiansø in the Central Baltic Sea. The clinical inspection revealed an open fracture of the right radius and ulna with necrosis of the surrounding tissue (Figure 1). The lesion was likely trauma-induced due to a clash with fishing-trawl. Other pertinent findings on the physical examination were a prominent keel and reduced pectoral musculature indicating starvation. The body weight was 864g, approximately 15-40% below normal body weight, without any embedded or freshly imprinted pellets.

Due to poor state, the gull was anesthetized using isoflurane delivered by facemask at 5%, the blood sample was drawn and subsequent IV pentobarbital (100 mg/kg; Euthasol 400 mg, Virbac Denmark) was administered for euthanasia. The following blood tests included hematology, plasma biochemistry and Serum Amyloid A (SAA). The avian and reptile estimated white blood cell count was performed using the Cornell University – Animal Health Diagnostic Center manual (Cornell, 2000). The analysis of biochemical parameters was conducted on an Abaxis Vetscan 2 using the VetScan® Avian Reptilian Profile Plus (Abaxis, Inc., USA) while a Eurolyser Solo analyser measured the SAA (Jacobsen et al., 2006; Hansen et al., 2006; Christensen et al., 2012; Gaillard et al., 2018). Reference values for herring gulls were not available for all parameters. Therefore, we used parameters derived from the *Laridae* family, i.e. laughing gulls for creatinine kinase (CK), potassium and sodium, while for hematology, we used values from black-backed gulls (Fox et al., 2007; ISIS, 2018).

Regarding SAA, no reference ranges for any healthy avian species is so far established, and falcon values were therefore used together with available mammalian data given levels of SAA is highly comparable across species (Averbeck, 1992; Hansen et al., 2006; Jacobsen et al., 2006; Fox et al., 2007; Christensen et al., 2012; Caliendo et al., 2013; Gaillard et al., 2018; ISIS, 2018). We acknowledge that reference ranges of clinical blood

Figure 1: Close up of the fractured radius and ulna with necrosis of the surrounding tissue. Christiansø, May, 2018.

parameters vary among avian species similar to what is seen in mammalian ones, disabling direct comparisons and interpretation in addition to influences from diurnal and environmental variation. However, for veterinary pathological interpretation of clinical blood parameters, abnormal values for sick individuals is often several folds above or below the typical healthy reference ranges for any species as seen, e.g. white blood cell Count (WBC) 46.25 being above all reference ranges (Table 1). We did not have permission to catch any healthy herring gulls at the eider colony for this study, while no more moribund gulls were found during the field study.

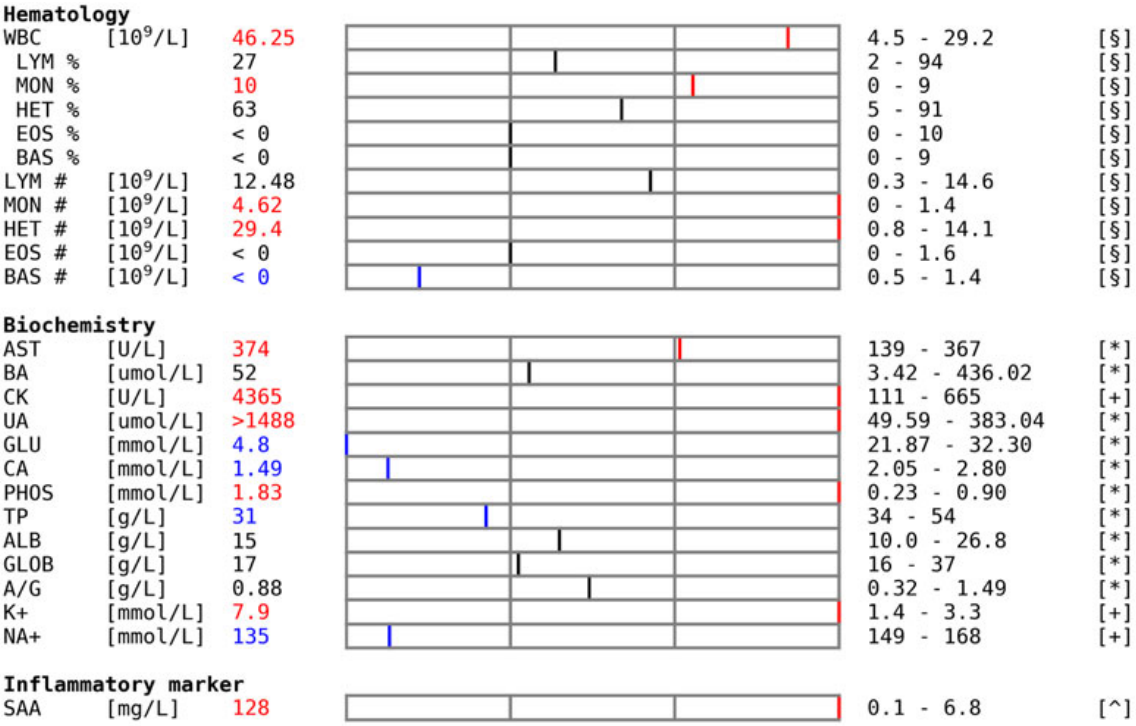
The results from the blood analysis are shown in Table 1. The manual slide estimate revealed a marked leucocytosis (46.25×10^9/L; reference interval 4.5-29.2), heterophilia (29.4×10^9/L; reference interval 0.8-14.1) and monocytosis (4.62×10^9/L; reference interval 0-1.4) (Averbeck 1992). A markedly increased activity of CK (4365 U/L; reference interval 111-665) and uric acid (>1488 umol/L; reference interval 49.59-383.04), in addition to hyperphosphatemia (1.83 mmol/L; reference interval 0.23-0.90), hyperkalemia (7.9 mmol/L reference interval 1.4-3.3) and hypoglycemia (4.8 mmol/L; reference interval 21.87-32.30) was also found (Fox et al., 2007; ISIS, 2018). Other clinically relevant abnormalities were a slightly lowered total protein (31 g/L; reference interval 34-54), slightly increased activity of aspartate transaminase (AST) (374 U/L; reference interval 139-367), moderate hyponatremia (135 mmol/L; reference interval 149-168), and moderate hypocalcemia (1.49; reference interval 2.05-2.80) and increased SAA of 128 mg/L (reference interval 0.1-6.8) (Fox et al., 2007; Caliendo et al., 2013; ISIS, 2018).

This report is the first description of the use of SAA in a moribund free-ranging gull spp. with a fractured wing. Combined with hematological and biochemical parameters, this biomarker might be a valuable tool to investigate the physiological status of wild avian species (Gruys et al., 2005; Garbus et al., 2019). The gull had an extreme hypoglycaemia of only 4.8 mmol/L (reference interval 21.87-32.30) which indicates starvation and sepsis (Hollmén et al., 2001; Thrall et al., 2012). A 6-fold increase in CK (4365 U/L; reference interval 111-665) suggested traumatic muscle lesions and fiber necrosis due to the catabolism of the protein as a consequence of starvation (Cherel et al., 1988; Thrall et al., 2012; Leissinger et al., 2017). The biochemical changes consistent with decreased glomerular filtration was reflected in the 4-fold increase in uric acid (>1488 umol/L; reference interval 49.59-383.04), moderate hyperphosphatemia (1.83 mmol/L; reference interval 0.23-0.90) and marked hyperkalemia (7.9 mmol/L reference interval 1.4-3.3) (Fox et al., 2007; ISIS, 2018) from pre-renal dehydration and renal insufficiency secondary to septicemia (Fox et al., 2007; Thrall et al., 2012; ISIS, 2018).

The moderate hyponatremia (135 mmol/L; reference interval 149-168) was likely due to renal losses (Lierz, 2003). The manual slide estimate revealed a marked leucocytosis (46.25×10^9/L; reference interval 4.5-29.2) with a marked heterophilia (29.4×10^9/L; reference interval 0.8-14.1) as well as monocytosis (4.62×10^9/L; reference interval 0-1.4) consistent with chronic active inflammation (Averbeck, 1992). A minimal elevation in AST (374 U/L; reference interval 139-367) with a normal bile acid indicates minimal liver affection (Lumeij, 2008).

SAA was tentatively measured to investigate further the inflammation noted on the haematology and plasma biochemistry of this herring gull. SAA is a positive acute phase protein that increases during systemic inflammation, thus being an integral part of the innate immune response in many vertebrates. In both human

Table 1: The hemogram, serum biochemistry, SAA, and electrolyte profile of the herring gull in question. In comparison, herring gull, laughing gull, and black-backed gull reference values are included. For SAA, falcons are used in comparison. Changes are marked in red (elevated) and blue (reduced).



*: Herring gull (Fox et al., 2007). +: Laughing gull (ISIS, 2018). ∧: Falcons (Caliendo et al., 2013). §: Black-backed gull (Averbeck, 1992).

and veterinary medicine, measurement of acute phase proteins provides valuable clinical information (Gruys et al., 2005; Jacobsen et al., 2006). The use of SAA has now been established in other species, e.g. dogs, cats, rabbits, cattle, horses, and fish, where the SAA usually is very low in concentration but increases dramatically with inflammation (Kimura et al., 1994; Nielsen et al., 2004; Christensen et al., 2012; Kania et al., 2014). The SAA concentrations in the blood of healthy animals range from <6.8 mg/L (falcons), >10 mg/L (dogs and cats), <20 mg/L (horses), which suggest comparable levels between species (Hansen et al., 2006; Jacobsen et al., 2006; Christensen et al., 2012; Caliendo et al., 2013; Gaillard et al., 2018).

The use of SAA as a parameter of inflammation in avian species is not well described, but SAA seems to be the most sensitive parameter in studies of chickens infected with bursal disease virus as well as other infectious diseases in chickens (Chamanza et al., 1999; Nazifi et al., 2010; Seifi et al., 2017). Caliendo et al. (2013), reported that SAA was significantly higher in falcons with inflammatory disease versus healthy falcons. The mean and standard deviation in the healthy group was 3.4±1.4 mg/L compared to 47.7±29.7 mg/L in the inflammatory group, while the maximum value was found to be 137.5 mg/L. As the SAA of this herring gull was 128 mg/L (reference interval based on falcons 0.1-6.8), it clearly supports the suspicion of septicemia.

The present case report is to our knowledge the first to use SAA in a case of a free-ranging herring gull. Using healthy falcons as reference is controversial but the only free-ranging avian reference available for healthy specimens. Indeed, this reference interval aligns with other recent avian references, mostly performed on healthy poultry control groups ranging <10 mg/L (Pirgozliev et al., 2019; Kaab et al., 2019). A previous study has described amyloidosis as a systemically distributed pathology of fibril structures in multiple organs, including liver, kidney, brain, and intestines in sick Baltic herring gulls using a very different analytic approach the present (Jansson et al., 2018). Further investigations should be undertaken regarding SAA use in free-ranging avian amyloidosis (Westermark and Westermark, 2009). It may be more frequent in polluted areas such as the Baltic Sea, likely because of environmental stressors increase the general state of inflammation.

Ethics Approval

The study was approved by the Danish Nature Agency and the Danish Ministry of Environment and Food (NST-304-0008). Blood samples and handling was conducted under permit no. 2017-15-0201-01205 (case no. 2017-15-0201-01205/MABJE) granted by the National Committee for the Protection of Animals used for Scientific Purposes.

Conclusions

In summary, this case provides knowledge about SAA in gulls and how bone fracture and secondary infection

may affect gull blood hematology and biochemistry. The SAA seems to be an important tool in the diagnosis of inflammation in avian species along with standard hematology testing. We encourage further studies on SAA in general and studies providing reference ranges in other domestic and wildlife avian species.

Article Information

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Conflict of Interest. The authors declare no conflict of interest.

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