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Hematology of Fledgling Manx Shearwaters (*Puffinus puffinus*) with and without 'Puffinosis'

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ABSTRACT: Hematological parameters were measured in 14 fledgling Manx shearwaters (*Puffinus puffinus*), with the disease puffinosis and in 10 birds that did not have the disease, on the Island of Skomer between 2 and 11 September 1991. The mean plasma fibrinogen concentration was significantly higher in the diseased birds and some of these had abnormally elevated monocyte counts. No other significant differences were observed.

Key words: Manx shearwater, *Puffinus puffinus*, hematology, puffinosis.

An epizootic has occurred annually in fledgling Manx shearwaters (*Puffinus puffinus*) in certain discrete parts of the islands of Skokholm and Skomer off the south west coast of Wales, United Kingdom (51°44'N, 5°19'W) (Brooke, 1990). The most prominent clinical sign is the development of large vesicles on the ventral and dorsal surfaces of the webs of the feet, but some birds also have conjunctivitis and locomotor disturbances; mortalty has been high (Dane et al., 1953; Harris, 1965). On web lesions, blisters form by accumulation of fluid which splits the surface keratinous layer from the epidermis of the web (Harris, 1965). The disease was named puffinosis by Miles and Stoker (1948) who first attempted to determine the etiology. Although there has been considerable interest in the epizootiology of the disease since it was first recorded in 1908 (Nuttall et al., 1982; Brooke, 1990), the cause remains unclear. Based on experimental transmission studies and the epizootiology, it appears that the disease may be an infection, possibly caused by an arthropod-borne virus (Brooke, 1990). Similar diseases have been reported in black-headed gulls (*Larus ridibundus*) (Jennings and Soulsby, 1958) and fulmars (*Fulmarus glacialis*) (MacDonald et al., 1967).

Our objective was to determine if there were hematological differences between birds with puffinosis, and those without the disease. As far as we are aware, apart from a report (Peirce, cited by Brooke, 1990) that a raised white cell count was observed in one out of six birds with puffinosis, no information has hitherto been available on the hematology of the Manx shearwater.

The study was carried out on the island of Skomer, 2 to 11 September 1991. Fledglings were readily caught for examination and blood sampling when they emerged from their nest burrows after dark. At this stage the birds were about 70 days of age (Nuttall et al., 1982). Samples were collected from 10 apparently healthy birds found within a few meters of the warden's house, in an area in which the disease does not occur, and from 14 birds that had the characteristic vesicles of puffinosis and were found in an area where the disease is known to occur. The stage of progression of the disease among affected birds could not be determined, but all had vesicles or recently burst vesicles at the time of sampling. Dane (1948) observed that blisters tend to develop in one to four days preceding death.

Blood samples (0.3 ml) were collected from the brachial vein, using 1 ml syringes fitted with 25 gauge needles. Smears were made and air-dried, and samples were immediately transferred to tubes containing ethylene diamine tetracetic acid (EDTA) to prevent clotting. These samples were transported as quickly as possible to a commercial laboratory (Ravenscourt Laboratories, Ravenscourt Square, London,

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TABLE 1. Hematology of Manx Shearwaters with and without puffinosis, on Skomer Island, Wales, September 1991.

Parameter	Apparently healthy birds			Puffinosis cases		
	Mean	SD or range	Number sampled	Mean	SD or range	Number sampled
Erythrocyte count ($\times 10^{12}$/l)	2.87	0.384	9	3.17	0.272	14
Hemoglobin (g/dl)	14.84	1.845	10	16.14	2.341	14
Hematocrit (l/l)	0.48	0.047	9	0.51	0.036	10
Mean corpuscular volume (fl)	166	13.7	9	161	6.9	10
Mean corpuscular hemoglobin (pg)	53.4	5.27	9	53.4	5.27	9
Mean corpuscular hemoglobin concentration (g/dl)	32.0	1.63	9	31.5	2.93	10
Thrombocytes ($\times 10^9$/l)	11.5	4.76	10	19.8	7.66	14
Total leukocyte count ($\times 10^9$/l)	5.39	4.262	10	5.04	3.083	14
Heterophils ($\times 10^9$/l)	2.81	2.531	10	2.31	1.547	14
Lymphocytes ($\times 10^9$/l)	1.19	2.244	10	1.35	1.163	14
Monocytes ($\times 10^9$/l)	0.25	0–0.87*	10	1.14	0.33–2.38*	14
Eosinophils ($\times 10^9$/l)	0.09	0–0.23*	10	0.09	0–0.20*	14
Basophils ($\times 10^9$/l)	0.29	0–0.76*	10	0.14	0–0.44*	14
Fibrinogen (g/l)	2.01	0.421	6	5.14	1.264	10

* Ranges rather than standard deviations are presented here because variation in these parameters was not normally distributed.

United Kingdom) for hematological examinations using the techniques of Hawkey and Samour (1988), and were examined in 24 to 48 hr after collection.

With the exception of monocyte, eosinophil, and basophil counts, which tend to show skewed distributions (Bennett et al., 1991a), the parameters studied were assumed to be normally distributed and were compared using a t-test appropriate for small, unequal samples (Sokal and Rohlf, 1981).

The means and ranges of most of the parameters examined were similar in both groups (Table 1). However, there was a significant difference in fibrinogen concentration ($t = 3.204$, $P > 0.01$) between the two groups (Table 1). The range in the apparently healthy birds was 1.67 to 2.75 g/l compared to 2.63 to 6.48 g/l in birds with puffinosis. Also, five of the birds with puffinosis had monocyte counts in excess of 1.32×10^9/l, the value listed by Hawkey (1991a) as the upper limit of the normal range seen in the Class Aves, and greater than the upper limit of the range among the normal shearwaters in our study.

The values for the hematological parameters measured in the healthy shearwaters were broadly consistent with findings in other species of birds (Bennett et al., 1991b). The red cell parameters, including mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were close to the means reported for birds by Hawkey (1991b).

Elevation in plasma fibrinogen level is a non-specific response associated with acute inflammation. It is a consistent response in birds and often occurs in bacterial infections (Hawkey and Hart, 1988). The significant increment in fibrinogen concentration in the birds with puffinosis was presumed to be associated with the web lesions although it is possible that other tissues were also involved.

Mean heterophil and total white cell concentrations were not elevated in the diseased compared with the healthy birds. This was surprising because birds typically develop a heterophilia in response to bacterial infections (Hawkey and Samour, 1988) and bacterial infections (which have been presumed to be secondary) appear to be common in shearwaters with puffinosis (Miles and Stoker, 1948).

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