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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Atypical Histiocytosis in Red Squirrels (*Sciurus vulgaris*)

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Summary

Four red squirrels (*Sciurus vulgaris*) were subjected to necropsy examination over a 3-year period as part of a broader surveillance study. The squirrels presented with cutaneous, subcutaneous and/or internal swellings and nodules that consisted microscopically of sheets of atypical round cells and multinucleated giant cells. There was moderate anisokaryosis with rare mitoses. Nuclei ranged from oval to indented or C-shaped and some were bizarre, twisted or multilobulated. Many giant cells also had a bizarre morphology, with anisokaryosis within individual cells. Giant cell nuclei were often multilobulated, ring-shaped or segmented. Affected internal organs varied depending on the squirrel, but included lymph node, kidney, intestinal tract and lungs. Representative lesions from each of the four squirrels were negative for acid-fast organisms. Formalin-fixed tissues from all four squirrels and ethanol-fixed tissue from one animal were negative for *Mycobacterium* by polymerase chain reaction. Immunohistochemically, the majority of mononuclear and multinucleated giant cells in all four squirrels strongly expressed vimentin and class II molecules of the major histocompatibility complex. Otherwise, the atypical mononuclear and multinucleated cells were negative for CD3, Pax-5, Mac387, CD18 and E-cadherin. Based on the combination of cellular morphology, arrangement and immunophenotype, a novel form of atypical histiocytosis is considered most likely in these squirrels, although the exact origin and triggering factors remain uncertain.

Keywords: histiocytosis; immunohistochemistry; red squirrel; *Sciurus vulgaris*

Two decades ago, Harris *et al.* (1995) described the extreme vulnerability of the red squirrel (*Sciurus vulgaris*) in the UK, concluding that the species was virtually extinct in most of England and Wales. A number of reports have since confirmed this decline, linking it to the introduction of the American grey squirrel (*Sciurus carolinensis*) (Rushton *et al.*, 2006). While direct competition for food and habitat is believed to play a role, much blame has been placed on squirrelpox virus, a member of the subfamily Chordopoxvirinae, which was seemingly introduced by the American grey squirrel (Thomas *et al.*, 2003; McInnes *et al.*, 2006). Native red squirrels are more vulnerable to infection than their grey counterparts and, since Scottish red squirrels account for three-quarters of the UK population, it is acknowledged that their conservation and a full understanding of their stressors are important (LaRose *et al.*, 2010). To that end, a necropsy examination-based survey has been underway at the University of Edinburgh for several years, focusing partly on the impact of squirrelpox infection on the red squirrel population, but also exploring the role of factors such as trauma, predation and starvation (LaRose *et al.*, 2010). During this larger study, an unusual presentation of skin disease was identified sporadically, characterized by proliferative lesions that also involved some internal organs.

Four female red squirrels were subjected to necropsy examination over a 3-year period

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(2012–2015) as part of the aforementioned surveillance study (LaRose *et al.*, 2010). All four originated from southwest Scotland and each was found dead with no prior clinical history. The squirrels presented with multiple soft to firm swellings and discrete nodules up to 15 mm in diameter. The skin and subcutis of the head were predominantly affected in three squirrels, particularly around the eyes, mandibular area, bridge of the nose, lips and chin (Fig. 1). On cut section, the nodules ranged from pink to brown and many were ulcerated or cavitated. The lungs of two squirrels contained multiple 1–2 mm diameter, tan to white nodules. Other more variably affected areas and internal organs are summarized in Table 1. A selection of organs was collected from each squirrel by the project's principal investigator (AM). Tissues were fixed in 10% neutral buffered formalin and processed routinely. Selected sections were stained with Gram and/or Ziehl–Neelsen (ZN) stains and sections from all four squirrels were further characterized by immunohistochemistry (IHC). Briefly, serial sections were dried at 37°C, incubated at 60°C, de-waxed, dehydrated and washed in Tris buffer. Endogenous peroxidase was blocked using Dako REAL peroxidase blocker (Dako, Ely, UK). All antibody incubations were performed for 30 min at room temperature using mouse monoclonal antibodies against vimentin (dilution 1 in 400; Novocastra, Milton Keynes, UK), CD3 (1 in 200; Leica, Milton Keynes, UK), feline CD18 (1 in 20; University of California at Davis, California, USA), Pax-5 (1 in 50; Becton Dickinson, Oxford, UK), E-cadherin (1 in 200; Becton Dickinson), Mac387 (1 in 600; Dako) and class II molecules of the major histocompatibility complex (MHCII; 1 in 60; Dako). Pretreatments comprised incubation for 15 min in high pH buffer (Vector Laboratories, Peterborough, UK) at 110°C for vimentin; 15 min in 0.01 M citrate buffer at 110°C for CD3, Pax-5, E-cadherin and MHCII; and 10 min in proteinase K for CD18 and Mac387. For all but CD18 and Mac387, 'visualization' was achieved using Envision anti-Mouse HRP (Dako) for 40 min followed by 3,3′-diaminobenzidine for 10 min. Visualization of CD18 and Mac387 was achieved using secondary ImmPRESS anti-mouse (Vector Laboratories) for 15 min. Squirrel lymph node served as positive control for all antibodies except for that against E-cadherin. Epidermis in the skin nodules served as a positive internal control for E-cadherin, but additional controls included canine colon and squirrel salivary gland. Negative controls comprised antibody diluent only (Dako).

Fig. 1. Red squirrel (*Sciurus vulgaris*) with centrally ulcerated skin nodules around the left eye (white and black arrowheads). Correlates with squirrel 3 in Table 1.

Possible mycobacterial infection was investigated by polymerase chain reaction (PCR). DNA was extracted from formalin-fixed and paraffin wax-embedded (FFPE) skin from three squirrels, ethanol-fixed skin from one squirrel and FFPE lung from another. The extraction method used for the FFPE tissues was described by Simpson *et al.* (2016). The same method was used for the ethanol-fixed tissue, omitting the xylene and ethanol washes. A pan-*Mycobacterium* spp. PCR directed against the heat shock protein (hsp) 65 gene was performed (Telenti *et al.*, 1993). Sections of ileum from a sheep with multibacillary paratuberculosis were used as extraction and positive PCR controls and sterile distilled water as a negative PCR control. Amplified DNA was excised from a 2% agarose gel, extracted using QIAquick PCR Purification Kit (Qiagen, Manchester, UK) and sequenced by MWG BioTech (Eurofins MWG Operon, Ebersberg, Germany).

Microscopically, the skin and subcutaneous nodules consisted of sheets of round cells with a moderate amount of smooth, pale eosinophilic cytoplasm and densely basophilic, generally eccentrically located, oval, indented or C-shaped nuclei. Some nuclei were bizarre, twisted or multilobulated. Nucleoli were variably distinct, anisokaryosis was moderate and mitotic figures were rare. Admixed throughout

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