Demodicosis in a captive African straw-coloured fruit bat (*Eidolon helvum*)

Running title: Demodicosis in an African fruit bat

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ABSTRACT

Demodicosis is most frequently observed in the domestic dog (*Canis familiaris*), but it has rarely been reported in bats (Chiroptera). The overpopulation of *Demodex* spp. that causes dermatological changes is generally associated with a compromised immune system. We describe the gross and histological features of generalized demodicosis in an adult female African straw-coloured fruit bat (*Eidolon helvum*) drawn from a captive research colony. The histology of the lesions revealed comedones and follicular infundubular cysts harbouring numerous *Demodex* spp. mites, eliciting a minimal inflammatory response in the adjacent dermis. The histological examination of a full set of tissues did not reveal clear evidence of immunosuppression, although a clinical history of recent

abortion and possible stressors due to captivity could be considered risk factors for the demodicosis. Attempts to determine the *Demodex* species using PCR on DNA extracted from the formalin fixed paraffin embedded tissue failed. This is the first clinical and histological description of demodicosis in *Eidolon helvum*.

Keyword: bat, *Demodex*, dermatology, veterinary pathology, wildlife diseases.

INTRODUCTION

Demodex mites (Acarina: Prostigmata: Demodicidae) are obligate host-specific mammalian ecto-parasites inhabiting the pilo-sebaceous units or the adjacent *stratum corneum* of the skin. Over 140 *Demodex* species have been identified in at least 11 orders of domestic and wild mammals, reflecting the constancy of the sheltered ecologic niche (Sastre et al. 2016). The mites' primary food source are follicular keratinocytes and the secretions of sebaceous glands (Gross et al. 2005; Mauldin et al. 2015). Low population densities of mites are considered commensals as part of the normal skin fauna (Gross et al. 2005; Mauldin et al. 2015). The host's immune system inhibits their proliferation, keeping mite numbers low in the absence of an inflammatory response (Gross et al. 2005; Mauldin et al. 2015). *Demodex* mite infestations usually remain asymptomatic, but these mites can be important causative agents for clinical dermatological disease, called demodicosis or demodectic mange.

Demodicosis is defined as a non-contagious parasitic inflammatory skin disease characterized by the presence of an overpopulation of *Demodex* spp. mites. The disease is often associated with immunodepression (a defect in the immune system), underlying immunosuppressive disease or debilitating conditions such as poor nutrition, stress, concurrent diseases or genetic predisposition. Demodicosis is distributed worldwide and is reported most frequently in domestic dogs and cats. In veterinary dermatology, canine and feline demodicosis may be divided into two forms based on the clinical manifestation: a generalized form, a potentially life-threatening disease, and a localized form, a mild self-limiting disease. The gross clinical features of canine demodicosis are typically small, well-circumscribed, erythematous, scaly, multifocal areas of alopecia that can coalesce, and sometimes form nodular lesions (Gross et al. 2005; Mauldin et al. 2015). The three histopathological patterns of inflammation are folliculitis and furunculosis, parafollicular granulomas, and lymphocytic mural interface folliculitis (Gross et al. 2005; Mauldin et al. 2015). All patterns may coexist, or individual patterns may predominate, and follicular

melanosis with pigmentary incontinence frequently occurs. In very rare cases, mites may greatly distend superficial hair follicles to form comedone-like lesions (Gross et al. 2005).

In other domestic species, apart from the goat and cattle in Africa, demodicosis is considered rare (Mauldin et al., 2015). The clinical appearance of demodicosis in goats is papulo-nodular, and histologically the nodules are follicular cysts lined by flattened squamous epithelium, filled with keratin scales and large numbers of demodicid mites. A mild inflammatory infiltrate may occur around the epithelial lining of affected hair follicles (Mauldin et al. 2015). Demodicosis has rarely been reported in bats (Chiroptera). Here we describe the gross and histological features of generalized demodicosis in an adult female African straw-coloured fruit bat (*Eidolon helvum*) drawn from a captive research colony.

MATERIAL AND METHODS

Clinical history of the animal and post mortem investigations

An adult female African straw-coloured fruit bat maintained within a group of 111 conspecifics for about 9 months, in a large flight cage (closed to public view) in the grounds of Accra Zoological Gardens in Achimota Forest Reserve, Accra, Ghana, was humanely euthanised on welfare grounds due to the presence of numerous cutaneous nodular lesions across the face and ventrum. The subject had been caught as part of a viral antibody dynamic study and tagged for individual identification following a protocol approved by the Zoological Society of London's Ethic Committee (Baker et al. 2014). Although pregnant when placed in the colony, the bat suffered an abortion after 5 months. Two months later a laceration to the leading-edge of the *patagium* was observed. The cutaneous nodular lesions were observed after a further 2 months, and the animal was euthanised subsequently.

A representative area of cutaneous lesions and samples from internal organs was fixed in formalin and paraffin embedded for routine histopathology (haematoxylin and eosin stained slides). Formalin-fixed, paraffin embedded sample of skin was subjected to xylene treatment. Total DNA isolation was performed using the QIAamp DNA Investigator kit (Qiagen). DNA was eluted in 30 µl of sterile water, quantified (Nanodrop 1000 Spectrophotometer) and stored at -80 °C until molecular analyses. Quality of the extraction was assessed by PCR amplification of two gene fragments of the mammal host. 12S rRNA and mitochondrial 16S rRNA gene fragments (expected fragments size: 200-250 bp) were amplified using universal PCR primers designed for the identification of vertebrate species (Kitano et al. 2006). The presence of *E. helvum* DNA was assessed in both PCR assays performed with universal vertebrate primers, the result was confirmed

by Sanger sequencing.

For the molecular identification of the mite, the PCR amplification was performed using both primers for species belonging to the genus *Demodex* and broad-range primers for the suborder Prostigmata. The PCR amplifications performed specifically for *Demodex* spp. were targeted on: i) a 340 bp 16S rRNA gene fragment (Zhao and Wu 2012); ii) a 429 bp *COXI* gene fragment (Zhao et al. 2013); a 230 bp 18S rDNA gene fragment (Zewe et al. 2017). The broad-range PCR amplification for the suborder Prostigmata targeted on a 500 bp 18S rDNA gene fragment (Otto and Wilson 2001). PCR amplifications were performed on undiluted and serially diluted DNA (from 1:10 to 1:1000) and possible inhibition of the amplification was also assessed.

RESULTS

The post-mortem examination revealed multiple nodular cutaneous lesions with asymmetrical distribution on the facial and ventral regions of the body. The dome-shaped nodules, 3-12 mm in diameter, were raised above the skin surface and were fluctuant, often with a central ostium (Figure 1). The other organs were grossly unremarkable. On histological examination, the cutaneous nodular lesions were found to comprise distended hair follicles (up to 4 mm in diameter) filled with numerous cigar-shaped arthropod parasites admixed with a scant amount of eosinophilic lamellar material consistent with flaky keratin (cystic comedonal appearance). These lesions elevated the epidermis, displacing and compressing adjacent dermis and adnexa. The arthropods were approximately 40 µm in diameter and 200 µm in length, with a body divided into three segments (a gnathosoma with mouthparts, a podosoma with short jointed appendages and a worm-shaped opisthosoma), and were characterized by an eosinophilic, chitinous, exoskeleton, a haemocoel, striated muscle, and digestive and reproductive tracts (Figure 2A,B). These histological features were consistent with those of a demodicid (*Demodex* sp.) mite.

The affected follicles were distended and lined by an attenuated infundibular epithelium, displaying keratohyalin granules. Distended follicles were surrounded by a thin rim of dense collagen with the rare presence of mononuclear inflammatory cells (Figure 2A). The adjacent dermis did not show inflammatory changes. Single parasites were rarely observed in hair follicles or in areas of the skin without associated changes close to the comedonal and cystic follicular lesions. Moderate follicular melanosis and a minimal pigmentary incontinence was present. Other histopathological findings included mild multifocal splenic follicular vascular hyalinosis, mild random multifocal chronic granulomatous hepatitis with minimal diffuse hepatocellular degeneration. Kidney sections displayed mild multifocal tubular mineralization and multifocal

minimal membrano-proliferative glomerular segmental changes. Other tissues (brain, adrenals, ovary, oviduct, urinary bladder, heart, lung, lymph nodes, abdominal fat) were unremarkable.

The amplification of DNA produced extremely weak bands at the expected molecular weight only for the undiluted sample, whereas the diluted samples did not show any positive amplification. No inhibition in the PCR reaction was highlighted. No amplification of *Demodex* DNA was achieved.

DISCUSSION

The growing awareness of bats as reservoir species for multiple infectious agents has increased interest in them as vectors of zoonotic pathogens (Mühldorfer et al. 2011; Baker et al. 2013, 2014). Old World fruit bats (family Pteropodidae) are a diverse group of non-echolocating bats that inhabit tropical regions in Africa, Asia, Australia, and many Pacific islands. In recent years bat species have been recognized as natural reservoir hosts for an increasing number of important zoonotic diseases, including those caused by filoviruses and paramyxoviruses (Baker et al. 2013). *Demodex* commensal parasitism in wild mammals is common, suggesting that this parasitic relationship is very ancient (Sastre et al. 2016). Fountain et al. (2017) investigated the epidemiology of skin lesions among captive bats, but without aetiological diagnosis. A small number of parasitological investigations have focused on the identification of commensal demodicid mites, including a report of infection in the New Zealand short-tailed bat (*Mystacina tuberculata*) in the absence of disease (Desch, 1989) and a description of demodicid mites from the eyelids of a Mexican bat (*Artibeus aztecus*) with blepharitis, but histological examination was not carried out (Vargas et al., 1995).

Demodex has been reported in fruit bats as a common commensal, but reports of demodicosis are rare. In the Egyptian fruit bat (*Rousettus aegyptiacus*), demodicosis has been reported, but has not been characterized clinically or described histologically (Childs-Sanford et al. 2009). In the big brown bat (*Eptesicus fuscus*), preputial *Demodex* sp. infestation has been described in the absence of gross lesions but in three out of four cases, folliculitis, dermatitis or preputial adenitis was diagnosed on histological examination (Lankton et al. 2013). A study on zoo-kept Egyptian fruit bats (*Rousettus aegyptiacus*) found an association between demodicosis and concurrent disease, supporting the hypothesis that predisposing factors causing immunosuppression lead to an increase in the *Demodex* sp. population and disease (Childs-Sanford et al. 2009). Among possible exogenous factors resulting in *Demodex* overgrowth are social and hierarchical stress (Childs-Sanford et al. 2009).

In our subject, although histopathology did not reveal morphological evidence suggestive of

impairment of the immune system, the clinical history of the animal revealed pregnancy, abortion and trauma, as well as capture from the wild and co-housing with new conspecifics, which are debilitating and predisposing factors that likely triggered changes in the immune status. In addition, the mild hepatic and nephropathic changes could have acted synergistically in debilitating the animal, making it more prone to the development of demodicosis. Parasitological characterization of the *Demodex* species present could not be conducted because no fresh tissue was available for parasitological examination (Mathison et al. 2014) and the weak positivity observed for the amplification of the host DNA suggested a high fragmentation of the extracted nucleic acid, usually due to overfixation (Rait et al. 2006; Dedhia et al. 2007). Considering the weak amplification of the vertebrate host DNA, the negative result for the amplification of the mite DNA could be thus attributed to the low quality of the available DNA template. The lack of amplification using PCR primer sets for both *Demodex* species and Prostigmata members occurred for short amplicons (around 230 bp) supported this hypothesis.

Conclusions

To the best of our knowledge, this report is the first to describe the gross and histological appearance of demodicosis in an African straw-coloured fruit bat (*E. helvum*). Demodicosis should be considered as a differential diagnosis in cases of cutaneous nodular and comedone-like lesions in this species and might be suggestive of non-specific stressors in affected individuals. More investigations are warranted to identify the species of *Demodex* spp. colonising *E. helvum* and possibly other species of fruit bats.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Figure captions

Figure 1 Adult female African straw-coloured fruit bat (*Eidolon helvum*): post mortem gross appearance of demodicosis: multifocal dome-shaped nodules 3-12 mm in diameter, raised above skin surface and fluctuant with frequently evident central follicular ostium.

Figure 2 Adult female African straw-coloured fruit bat (*Eidolon helvum*), haired skin. (A) Low magnification appearance of extremely dilated, cystic hair follicle comedones with numerous intralesional demodicid mites, evocating a minimal perifollicular inflammatory response. (haematoxylin and eosin stained slide; objective: 10×). (B) Detail of adult *Demodex* spp., elongated cigar-shaped parasites dwelling in the dilated hair follicle. (haematoxylin and eosin stained slide, objective: 40×).

FIG 1

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New Figure 2AB

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