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2 1 **Demodicosis in a captive African straw-coloured fruit bat (*Eidolon***
3
4 2 ***helvum*)**

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8 4 **Running title:** Demodicosis in an African fruit bat
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47 25 **ABSTRACT**

48 26 Demodicosis is most frequently observed in the domestic dog (*Canis familiaris*), but it has rarely
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50 27 been reported in bats (Chiroptera). The overpopulation of *Demodex* spp. that causes dermatological
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52 28 changes is generally associated with a compromised immune system. We describe the gross and
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54 29 histological features of generalized demodicosis in an adult female African straw-coloured fruit bat
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56 30 (*Eidolon helvum*) drawn from a captive research colony. The histology of the lesions revealed
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58 31 comedones and follicular infundibular cysts harbouring numerous *Demodex* spp. mites, eliciting a
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60 32 minimal inflammatory response in the adjacent dermis. The histological examination of a full set of
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62 33 tissues did not reveal clear evidence of immunosuppression, although a clinical history of recent
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1 abortion and possible stressors due to captivity could be considered risk factors for the demodicosis.
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 3 Attempts to determine the *Demodex* species using PCR on DNA extracted from the formalin fixed
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 5 paraffin embedded tissue failed. This is the first clinical and histological description of demodicosis
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 7 in *Eidolon helvum*.

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

12 13 14 15 16 INTRODUCTION

17
 18 *Demodex* mites (Acarina: Prostigmata: Demodicidae) are obligate host-specific mammalian ecto-
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 20 parasites inhabiting the pilo-sebaceous units or the adjacent *stratum corneum* of the skin. Over 140
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 22 *Demodex* species have been identified in at least 11 orders of domestic and wild mammals,
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 24 reflecting the constancy of the sheltered ecologic niche (Sastre et al. 2016). The mites' primary food
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 26 source are follicular keratinocytes and the secretions of sebaceous glands (Gross et al. 2005;
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 28 Mauldin et al. 2015). Low population densities of mites are considered commensals as part of the
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 30 normal skin fauna (Gross et al. 2005; Mauldin et al. 2015). The host's immune system inhibits their
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 32 proliferation, keeping mite numbers low in the absence of an inflammatory response (Gross et al.
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 34 2005; Mauldin et al. 2015). *Demodex* mite infestations usually remain asymptomatic, but these
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 36 mites can be important causative agents for clinical dermatological disease, called demodicosis or
 37
 38 demodectic mange.

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 40 Demodicosis is defined as a non-contagious parasitic inflammatory skin disease
 41
 42 characterized by the presence of an overpopulation of *Demodex* spp. mites. The disease is often
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 44 associated with immunodepression (a defect in the immune system), underlying
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 46 immunosuppressive disease or debilitating conditions such as poor nutrition, stress, concurrent
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 48 diseases or genetic predisposition. Demodicosis is distributed worldwide and is reported most
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 50 frequently in domestic dogs and cats. In veterinary dermatology, canine and feline demodicosis may
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 52 be divided into two forms based on the clinical manifestation: a generalized form, a potentially life-
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 54 threatening disease, and a localized form, a mild self-limiting disease. The gross clinical features of
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 56 canine demodicosis are typically small, well-circumscribed, erythematous, scaly, multifocal areas of
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 58 alopecia that can coalesce, and sometimes form nodular lesions (Gross et al. 2005; Mauldin et al.
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 60 2015). The three histopathological patterns of inflammation are folliculitis and furunculosis,
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 62 parafollicular granulomas, and lymphocytic mural interface folliculitis (Gross et al. 2005; Mauldin
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 64 et al. 2015). All patterns may coexist, or individual patterns may predominate, and follicular
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1 melanosis with pigmentary incontinence frequently occurs. In very rare cases, mites may greatly
 2 distend superficial hair follicles to form comedone-like lesions (Gross et al. 2005).

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

11 MATERIAL AND METHODS

12 Clinical history of the animal and post mortem investigations

13 An adult female African straw-coloured fruit bat maintained within a group of 111 conspecifics for
 14 about 9 months, in a large flight cage (closed to public view) in the grounds of Accra Zoological
 15 Gardens in Achimota Forest Reserve, Accra, Ghana, was humanely euthanised on welfare grounds
 16 due to the presence of numerous cutaneous nodular lesions across the face and ventrum. The subject
 17 had been caught as part of a viral antibody dynamic study and tagged for individual identification
 18 following a protocol approved by the Zoological Society of London's Ethic Committee (Baker et al.
 19 2014). Although pregnant when placed in the colony, the bat suffered an abortion after 5 months.
 20 Two months later a laceration to the leading-edge of the *patagium* was observed. The cutaneous
 21 nodular lesions were observed after a further 2 months, and the animal was euthanised
 22 subsequently.

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

1 by Sanger sequencing.

2 For the molecular identification of the mite, the PCR amplification was performed using
 3 both primers for species belonging to the genus *Demodex* and broad-range primers for the suborder
 4 Prostigmata. The PCR amplifications performed specifically for *Demodex* spp. were targeted on: i)
 5 a 340 bp 16S rRNA gene fragment (Zhao and Wu 2012); ii) a 429 bp *COXI* gene fragment (Zhao et
 6 al. 2013); a 230 bp 18S rDNA gene fragment (Zewe et al. 2017). The broad-range PCR
 7 amplification for the suborder Prostigmata targeted on a 500 bp 18S rDNA gene fragment (Otto and
 8 Wilson 2001). PCR amplifications were performed on undiluted and serially diluted DNA (from
 9 1:10 to 1:1000) and possible inhibition of the amplification was also assessed.

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RESULTS

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

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

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

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

7 **DISCUSSION**

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

21 *Demodex* has been reported in fruit bats as a common commensal, but reports of
22 demodicosis are rare. In the Egyptian fruit bat (*Rousettus aegyptiacus*), demodicosis has been
23 reported, but has not been characterized clinically or described histologically (Childs-Sanford et al.
24 2009). In the big brown bat (*Eptesicus fuscus*), preputial *Demodex* sp. infestation has been
25 described in the absence of gross lesions but in three out of four cases, folliculitis, dermatitis or
26 preputial adenitis was diagnosed on histological examination (Lankton et al. 2013). A study on zoo-
27 kept Egyptian fruit bats (*Rousettus aegyptiacus*) found an association between demodicosis and
28 concurrent disease, supporting the hypothesis that predisposing factors causing immunosuppression
29 lead to an increase in the *Demodex* sp. population and disease (Childs-Sanford et al. 2009). Among
30 possible exogenous factors resulting in *Demodex* overgrowth are social and hierarchical stress
31 (Childs-Sanford et al. 2009).

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

27 **Conclusions**

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

42 **Declaration of conflicting interests**

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

47 **Funding**

48 This work was part-funded by a Wellcome Trust Research Training Fellowship (KSB) and KSB is
 49 supported by a Wellcome Trust Clinical Career Development Fellowship (106690/Z/14/Z).

52 **Acknowledgements**

53 The authors thank Richard Irvine for his constructive review of the manuscript.

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42 **Figure captions**

43 **Figure 1** Adult female African straw-coloured fruit bat (*Eidolon helvum*): post mortem gross
44 appearance of demodicosis: multifocal dome-shaped nodules 3-12 mm in diameter, raised above
45 skin surface and fluctuant with frequently evident central follicular ostium.
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50 **Figure 2** Adult female African straw-coloured fruit bat (*Eidolon helvum*), haired skin. (A) Low
51 magnification appearance of extremely dilated, cystic hair follicle comedones with numerous
52 intralesional demodicid mites, evocating a minimal perifollicular inflammatory response.
53 (haematoxylin and eosin stained slide; objective: 10×). (B) Detail of adult *Demodex* spp., elongated
54 cigar-shaped parasites dwelling in the dilated hair follicle. (haematoxylin and eosin stained slide,
55 objective: 40×).
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