

## R.H: STAPHYLOCOCCI IN CAPTIVE FRUIT BATS

### DIVERSITY OF STAPHYLOCOCCAL SPECIES CULTURED FROM CAPTIVE LIVINGSTONE'S FRUIT BATS (*PTEROPUS LIVINGSTONII*) AND THEIR ENVIRONMENT

5 Kay Fountain B.Vet.Med., M.V.S. Larry Roberts B.V.M.&S., Ph.D., Victoria Young M.Sc., Alberto  
Barbon Ldo.Vet., Cert.Zoo.Med., Sian-Marie Frosini B.Vet.Med., Ph.D., David H. Lloyd B.Vet.Med.,  
Ph.D., Dipl., E.C.V.D., Anette Loeffler Dr.Med.Vet., Ph.D., Dipl., E.C.V.D.

From the:

Dept of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY

10 (Fountain). Idexx Laboratories, Grange House, Sandbeck Way, Wetherby LS22 7DN (Roberts,  
Young). Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, AL9 7TA (Loeffler,  
Lloyd, Frosini). Jersey Zoo, La Profonde Rue, Trinity, JE3 5BP Jersey, Channel Islands (Barbon).

Corresponding author; Kay Fountain, [kif21@bath.ac.uk](mailto:kif21@bath.ac.uk)

15

20

25

Abstract: Livingstone's fruit bats (*Pteropus livingstonii*) are Critically Endangered and a captive population has been established as part of the IUCN Species Action Plan. The largest colony, in Jersey Zoo, was sampled for staphylococcal carriage and at infection sites, as disease associated with staphylococci had previously been found. Staphylococci were

30 cultured from swabs from 44 bats (skin, oropharynx, mouth ejecta, skin lesions) and from their enclosure. The isolates were identified by MALDI-TOF; antimicrobial susceptibility testing was performed by disc diffusion and screening for *mecA* and *mecC*. Seventeen species of coagulase-negative staphylococci including *Staphylococcus xylosum*, *S. kloosii*, *S. nepalensis* and *S. simiae* were isolated. *Staphylococcus aureus* was identified from both

35 carriage and lesional sites. These findings suggest *S. nepalensis* may be part of the normal carriage flora of bats. Antimicrobial resistance rates were low and MRSA was not identified. Sampling of mouth ejecta for staphylococci may provide results representative for carriage sites.

Keywords: Livingstone's fruit bat, MALDI-TOF, skin lesion, Staphylococci, *S. simiae*.

40

## BRIEF COMMUNICATION

Conservation interventions to assist endangered species of wildlife often involve moving  
45 individuals from one place to another, sometimes with a period of captivity which may be very brief  
or encompass many generations.<sup>15</sup> It is important for the success of the intervention that the individual  
remains healthy and fit for eventual release, but also that the normal suite of colonising  
microorganisms associated with that individual remains unchanged.<sup>6</sup>

At Jersey Zoo (JZ) (Jersey, CI) as part of an International Union for Conservation of Nature  
50 (IUCN) Species Action Plan, a breeding colony of the Critically Endangered Livingstone's fruit bats  
(*Pteropus livingstonii*) was established between 1993 and 1995 by the capture of 17 wild bats from  
their native Comoros Islands.<sup>9,17</sup> Since then this founder population has bred, descendants have been  
transferred to, and returned from other zoos, principally Bristol Zoological Gardens, UK (BZG), and a  
group of around 45 bats was present at JZ during the study period.

55 Anecdotal clinical reports from JZ and BZG for the years 1993 to 2013 suggested that  
*Staphylococcus aureus* and other staphylococci had been involved in skin, soft tissue, bone and  
internal infections, some of which had resulted in death or euthanasia. Staphylococci are opportunistic  
pathogens and, in humans and dogs, it has been shown that the majority of infections involve  
endogenous strains that are carried by the infected individual on skin or mucosae.<sup>16,21</sup> Staphylococcal  
60 carriage is well documented in domesticated animal species but only in a few wildlife hosts.<sup>3,7</sup>

This study characterises the species and antimicrobial resistance patterns of staphylococci isolated  
from healthy and lesional skin, pharynx and mouth ejecta of captive Livingstone's fruit bats, and from  
environmental surfaces in their enclosure.

65 This study was approved by the Royal Veterinary College Clinical Research Ethical Review Board  
(CRERB URN 2015 1332). Livingstone's fruit bats and their enclosure at JZ (one large flight tunnel  
with a separate small hospital enclosure), were sampled on three occasions between 2014 and 2016.<sup>22</sup>  
Bats were sampled opportunistically by swabbing healthy ventral wing skin, oropharyngeal mucosae

in bats anaesthetised for other purposes, mouth ejecta and skin lesions, using cotton swabs dipped in tryptic soy broth (Oxoid™, Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, RG24 8PW, UK) with 10% salt (Sigma-Aldrich, Gillingham, Dorset, SP8 4XT, UK) (TSB+). Environmental sites (food cups, ropes, flooring) were sampled by rubbing a TSB+-moistened cotton swab over an area approximately 3 cm<sup>2</sup> just before daily enclosure cleaning.

Swabs were incubated in TSB+, plated onto 5% sheep blood agar and up to five morphologically distinct staphylococcal-type colonies were tested for coagulase production by the slide coagulase test using rabbit plasma (Pro-Lab Diagnostics, Wirral, CH62 3QL, UK). Coagulase-negative isolates (CoNS) were identified using matrix assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF) (Idexx, Wetherby, LS22 7DN, UK). Antimicrobial susceptibility was determined in all isolates by disc diffusion for oxytetracycline (30 mg), amoxicillin (10 mg), clindamycin (2 mg), cephalexin (30 mg), enrofloxacin (5 mg), trimethoprim-sulfamethoxazole (25 mg), and in the CoNS for cefoxitin (30 mg) as an indicator for methicillin susceptibility (all discs from Oxoid™). Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints were used as for CoNS and *S. aureus* from animals where available, otherwise as described by Carson (2012).<sup>3,5</sup> *S. aureus* isolates were confirmed by PCR demonstration of the *nuc* gene, their resistance to methicillin was screened using agar containing 2 mg/l oxacillin (ORSAB, plus selective supplement, Oxoid™) and the presence of *mecA* and *mecC* was determined in their genome sequences which had been obtained using Illumina Miseq sequencing (Illumina, California, 92122, US) as part of another continuing study.

A total of 91 swabs was taken from 44 different bats and from 19 environmental sites. Samples from bats included 62 swabs from carriage sites (skin n=42, oropharynx n=20), six from mouth ejecta and four from skin lesions. Swabs yielded 213 presumed staphylococcal isolates; of these, 145 were confirmed as belonging to seventeen species of CoNS by MALDI-TOF (Table 1). Four isolates were not *Staphylococcus*, five failed to regrow, 29 were below the manufacturer's cut-off point for MALDI-TOF identification and were excluded, and 30 coagulase-positive isolates were confirmed as *S. aureus*.

The skin lesions sampled in four individuals were: i) superficial acute necrotic dermatitis involving both wings of one bat, ii) superficial crusting dermatitis of an ear pinna, iii) deep pyoderma of a rostral mandible including osteomyelitis, and iv) an infected wing wound.

100 None of the *S. aureus* isolates showed phenotypic resistance to methicillin (screening agar) and none was found to carry *mecA* or *mecC*. All staphylococci were broadly susceptible to antimicrobials with only one isolate showing resistance to four antimicrobial classes, three isolates to three classes, and the remaining isolates to one or two classes. Resistance occurred most commonly to amoxicillin (n = 68, 38.9%), and 11 CoNS isolates were resistant to cefoxitin (7.6%).

105 Studies using the 16s rRNA gene to compare the skin microbiome of captive fruit bats and free-ranging insectivorous bats in different sites, and captive and free-ranging Tasmanian devils (*Sarcophilus harrisii*) have found that both the host species and the environment are important factors in determining the composition of the skin flora.<sup>2,4,12</sup> However, these studies did not specifically address the impact of pathogens such as staphylococci in captive populations. Staphylococcal faecal carriage in free-ranging straw-coloured fruit bats, and pharyngeal carriage in several bat species in 110 Gabon have been described but this is the first report of the carriage of staphylococci on the wing, in the oropharynx and mouth ejecta of captive Livingstone's fruit bats, and of the staphylococci found in their enclosure.<sup>1,8</sup>

The data from this study show that *S. xylosus*, *S. nepalensis*, *S. saprophyticus*, and *S. aureus* were 115 commonly present, and two species not previously described on bat skin were found: *S. nepalensis*, and *S. simiae*. *S. aureus* isolates were recovered from each of the four lesional swabs, suggesting that, as in other host species, this species has a significant role in skin and soft tissue infections in bats.

Sampling six mouth ejecta as described here yielded a substantial number of staphylococci which suggests this may provide representative results non-invasively and warrants further study.

120 Of the three species not previously identified from bats, *S. nepalensis* has been described in pneumonia in goats, in human clinical material, fermented fish, and associated with mature bat guano.<sup>13,18,20,23</sup> It has rarely been reported in mammalian skin carriage, but it is possible that in

previous reports it may have been misidentified as *S. xylosus* which is phenotypically nearly identical.<sup>13</sup> *S. simiae* was first isolated from the gastrointestinal tract of South American squirrel monkeys (*Saimiri sciureus*) and genomic analysis revealed a close relationship to *S. aureus*.<sup>14,19</sup> It has been isolated from fish and has been found to produce virulence factors but is otherwise little described.<sup>11</sup> *S. succinus* was only found in the environment in this study, so it cannot be definitively associated with Livingstone's bats. However, it was the predominant species recovered from small wild mammals in Europe, and vancomycin resistant isolates were recovered from free-ranging songbirds in America.<sup>7,10</sup>

In this colony of captive Livingstone's bats, the overall level of antimicrobial resistance was surprisingly low and is in contrast to other wildlife studies.<sup>3</sup> Clavulanic acid potentiated amoxicillin is the first line antimicrobial for sick bats in the colony, which may explain the finding of some resistance to amoxicillin in this study.

As in other host species, many different staphylococcal species were found on carriage sites of captive Livingstone's bats but *S. aureus* dominated on lesional skin. This suggests a similar opportunistic aetiology of staphylococcal skin infections as in other hosts and warrants investigations into bacterial complications when injuries or disease occur. The unique epidemiological setting of these captive bats provides further opportunity to study the origin and evolution of staphylococci in the context of some contact with humans and other wild and domestic animals.

Acknowledgments: The authors would like to thank all staff at JZ especially Dominic Wormell, Gayle Glendewar, Edward Bell, Ann Thomasson, and Andrew Routh, and Ben Pascoe at University of Bath for genome sequencing.

Funding: This study was funded by a training grant from the European Society of Veterinary Dermatology. Grant number ESVD 3577.

146

147

## LITERATURE CITED

- 150 1. Akobi B, Aboderin O, Sasaki T, Shittu A. Characterization of *Staphylococcus aureus* isolates from faecal samples of the Straw-Coloured fruit bat (*Eidolon helvum*) in Obafemi Awolowo University (OAU), Nigeria. BMC Microbiol. 2012;12:279. doi: 10.1186/1471-2180-12-279
2. Avena CV, Parfrey LW, Leff JW, Archer HM, Frick WF, Langwig KE, Kilpatrick AM, Powers KE, Foster JT, McKenzie VJ. Deconstructing the bat skin microbiome: Influences of the host  
155 and the environment. Front Microbiol. 2016;7:1753. doi: 10.3389/fmicb.2016.01753
3. Carson M, Meredith AL, Shaw DJ, Giotis ES, Lloyd DH, Loeffler A. Foxes as a potential wildlife reservoir for *mecA*-positive staphylococci. Vector Borne Zoonotic Dis. 2012;12(7):583-587. <https://doi.org/10.1089/vbz.2011.0825>
4. Cheng Y, Fox S, Pemberton D, Hogg C, Papenfuss AT, Belov K. The Tasmanian devil  
160 microbiome-implications for conservation and management. Microbiome 2015;3(1):76 <https://doi.org/10.1186/s40168-015-0143-0>
5. Clinical and Laboratory Standards Institute. Performance standards from antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard - fourth edition. Clinical and Laboratory Standards Institute document VET01-A4. Clinical and Laboratory  
165 Standards Institute, Wayne, PA; 2013.
6. Ewen JG, Armstrong DP, Empson R, Jack S, Makan T, McInnes K, Parker KA, Richardson K, Alley M. Parasite management in translocations: lessons from a threatened New Zealand bird. Oryx. 2012;46(3):446-456. <https://doi.org/10.1017/S0030605311001281>
7. Hauschild T, Slizewski P, Masiewicz P. Species distribution of staphylococci from small  
170 wild mammals. Syst Appl Microbiol. 2010;33(8):457-460. <https://doi.org/10.1016/j.syapm.2010.08.007>
8. Held J, Gmeiner M, Mordmüller B, Matsiégui P-B, Schaer J, Eckerle I, Weber N, Matuschewski K, Bletz S, Schaumburg F. Bats are rare reservoirs of *Staphylococcus aureus* complex in Gabon. Infect Genet Evol. 2017;47(1):118-120. <https://doi.org/10.1016/j.meegid.2016.11.022>
- 175 9. International Union for Conservation of Nature. Species action plan for Livingstone's fruit

bat (Jersey CI, Durrell Wildlife Conservation Trust). 1995 [Accessed 2018 June 7]; Available at:  
<https://portals.iucn.org/library/node/7368>.

10. Ishihara S, Bitner JJ, Farley GH, Gillock ET. Vancomycin-resistant gram-positive cocci isolated from the saliva of wild songbirds. *Curr Microbiol*. 2013;66(4): 337-343.

180 <https://doi.org/10.1007/s00284-012-0278-1>

11. Kaito C, Usui K, Kyuma T, Sekimizu K. Isolation of mammalian pathogenic bacteria using silkworms. *Drug Discov Ther*. 2011;5(2):66-70. doi: 10.5582/ddt.2011.v5.2.66

12. Lemieux-Labonté V, Tromas N, Shapiro BJ, Lapointe FJ. Environment and host species shape the skin microbiome of captive neotropical bats. *PeerJ*. 2016;4:e2430. doi: 10.7717/peerj.2430

185 13. Nováková D, Pantůček R, Petráš P, Koukalová D, Sedláček I. Occurance of *Staphylococcus nepalensis* strains in different sources including human clinical material. *FEMS Microbiol Lett*. 2006;263(2):163-168. <https://doi.org/10.1111/j.1574-6968.2006.00408.x>

14. Pantůček R, Sedláček I, Petráš P, Koukalová D, Švec P, Štětina V, Vancanneyt M, Chrastinová L, Vokurková J, Růžičková V, Doškař J, Swings J, Hájek V. *Staphylococcus simiae* sp nov., isolated from South American squirrel monkeys. *Int J Syst Evol Microbiol*. 2005;55(5):1953-1958. <https://dx.doi.org/10.1099/ijs.0.63590-0>

15. Parker KA, Dickens MJ, Clarke RH, Lovegrove TG. The theory and practice of catching, holding, moving and releasing animals, In: J. Ewen, D. Armstrong, K. Parker, P. Seddon, Editors, *Reintroduction biology: integrating science and management*. Chichester: Wiley-Blackwell; 2012. p 195 105-137.

16. Pinchbeck LR, Cole LK, Hillier A, Kowalski JJ, Rajala-Schultz NJ, Bannerman TL, York S. Genotypic relatedness of staphylococcal strains isolated from pustules and carriage sites in dogs with superficial bacterial folliculitis. *Am J Vet Res*. 2006;67(8):1337-1346. <https://doi.org/10.2460/ajvr.67.8.1337>

200 17. Sewall B. *Pteropus livingstonii*. The International Union for Conservation of Nature Red List of Threatened Species 2016. [accessed 2018 June 7] e.T18732A22081502. (IUCN). <http://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T18732A22081502.en>.

18. Spergser J, Wieser M, Taubel M, Rossello-Mora RA, Rosengarten R, Busse HJ.



- 205 *Staphylococcus nepalensis* sp nov., isolated from goats of the Himalayan region. Int J Syst Evol  
Microbiol. 2003;53(6):2007-2011. <https://dx.doi.org/10.1099/ijss.0.02646-0>
19. Suzuki H, Lefébure T, Bitar PP, Stanhope MJ. Comparative genomic analysis of the genus  
*Staphylococcus* including *Staphylococcus aureus* and its newly described sister species  
*Staphylococcus simiae*. BMC Genomics 2012;13(1):38. doi: 10.1186/1471-2164-13-38.
- 210 20. Vandžurová A, Bačkor P, Javorský P, Pristaš P. *Staphylococcus nepalensis* in the guano of  
bats (Mammalia). Vet Microbiol. 2013;164(1-2):116-121.  
<https://doi.org/10.1016/j.vetmic.2013.01.043>
21. Wertheim HFL, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA,  
Nouwen JL. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis.  
2005;5(12):751-762. [https://doi.org/10.1016/S1473-3099\(05\)70295-4](https://doi.org/10.1016/S1473-3099(05)70295-4)
- 215 22. Wormell D. The recycled roost. In: *Zooquaria* (European Association of Zoos and  
Aquaria), 2012 [accessed 2018 June 7];80:22-26. Available at;  
<http://interactivepdf.uniflip.com/2/48142/290257/pub/html/22.html>.
- 220 23. Zhang H, Li Y, Xu K, Wu J, Dai Z. Microbiological changes and biodiversity of cultivable  
indigenous bacteria in Sanbao larger yellow croaker (*Pseudosciaena crocea*), a Chinese salted and  
fermented seafood. J Food Sci. 2015;80(4):M776-M781. doi: 10.1111/1750-3841.12818.