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| **TITLE OF CASE**  |
|  Diseases and histopathological findings from lesional pinnae of 10 bats  |
| **SUMMARY**  |
|  Pinnae are vital structures for echolocation and survival in many species of bats. Pinnal lesions have been described as common in captive bats, but information on specific diseases affecting the pinnae is scarce. A collection of histological sections of lesional pinnae from 10 bats, archived by bat conservation centers worldwide, was examined to identify potential aetiologies of disease. No neoplastic changes were seen but all pinnae showed inflammation, most commonly intraepidermal pustular dermatitis, deep perivascular dermatitis, nodular and/or diffuse dermatitis, and panniculitis. All bats with deep inflammation had concurrent pinnal perichondritis and cartilage necrosis. Microbial overgrowth was frequent, and pyoderma was confirmed in two bats based on histopathological and culture findings. Keratinocytes with features characteristic of koilocytes were noted in five bats, however immunohistochemistry would be required to confirm viral infection. Although no single aetiological agent was identified, deep inflammation was common and severe highlighting the potential detriment to affected bats and the need for future study of these delicate structures.   |
| **BACKGROUND**  |
|  Bats, the only mammals capable of winged flight, have key roles in global insect control, plant pollination and seed dissemination.1 Worldwide, bat numbers are decreasing,2 with 16 percent of Chiroptera species listed as critically endangered, endangered or vulnerable on the International Union for Conservation of Nature Red List.3 Habitat loss, climatic change, human-bat conflict and predation are well-recognized threats to bat species,1 but little is known about the impact of diseases on bats, other than those with zoonotic potential.4 In a post-mortem study of 486 bats in Germany, fatal bacterial and viral infections and parasitic infestations were identified in 12% of them, highlighting the potentially catastrophic impact that infectious or parasitic agents may have on wild bats.5 Skin disease in particular, was found to be common in a recent international survey of captive bat populations;one third of skin lesions were reported to affect bat pinnae and frequently involved tissue necrosis.6 Since many species of bats utilize echolocation for hunting, predator avoidance and social interaction, pinnal health can be crucial for bat welfare and survival. As large, thin and sparsely haired structures, bat pinnae are naturally vulnerable to trauma and temperature extremes, but also to any primary or secondary disease processes affecting skin. Although histology of normal bat pinnae has revealed the layers and adnexa expected for haired mammalian skin,7-9 histopathology from lesional pinnae has rarely been described7,10,11and might provide further insight into diseases affecting these delicate but vital appendages. |
| **CASE PRESENTATION** |
|  A collection of histopathological specimens from lesional pinnae of 10 bats was retrospectively examined, and analysed for predominant histopathological patterns of inflammation and to propose potential aetiologies for pinnal disease. Pinnal biopsies had been taken between 2006 to 2017 from 10 free-ranging and captive bats (table 1), including one *Eptesicus serotinus* bat (Serotine bat, case 1) from the Stapeley Grange Wildlife Centre RSPCA (UK), five *Mystacina tuberculata* bats *(*NZ lesser short-tailed bats, case 2-6) from the Kapiti Island translocation project (New Zealand), one *Pteropus alecto* bat (Black flying fox, case 7) from the Australian Registry of Wildlife Health, one *Pteropus livingstonii* bat (Livingstone’s fruit bat, case 8), from Jersey Zoo (UK), one *Macroderma gigas* bat (Ghost bat, case 9) from the Australian Registry of Wildlife Health and one *P. livingstonii* bat (case 10) from Bristol Zoo (UK). Lesional pinnae had been sampled through biopsies for diagnostic purposes at the time that bats had presented with skin disease or at post-mortem examination. In addition, non-lesional pinnae from two wildlife casualty bats, a *Plecotus auritus* bat(Brown long-eared bat, case 11) and a *E. serotinus* bat (case 12) from Hurstpierpoint Bat Hospital (UK), that had been obtained post-mortem, were available for reference. Examination of specimens had been approved by the Royal Veterinary College Clinical Research Ethical Review Board (CRERB) as part of an international survey on bat skin diseases (URN 2015 1332).6 As for most wildlife, information on signalment was limited (table 1) and gross pinnal morphology was non-specific (figure 1A) or unavailable. At least one haematoxylin and eosin (H&E) slide was available for each bat and a selection of Gram, Periodic Acid-Schiff (PAS) or Ziehl-Neelsen (ZN) stained slides available for nine of 10 bats.  |
| **INVESTIGATIONS** |
|  Slides were examined by three of the authors (JB, JW, AL) and information subsequently merged (JB) or discussed for consensus when indicated. All specimens examined included auricular cartilage with overlying skin. Salient dermato-histopathological features were described, including patterns of cutaneous inflammation,12,13 inflammatory cell type, and location and morphology of microbes. Inflammation was seen in all ten pinnae and more than one inflammatory pattern was seen in 9/10 cases. An intraepidermal pustular dermatosis (figure 1B) was identified in six cases and pustules contained either neutrophils or a mixed inflammatory infiltrate (table 2). Nine of 10 bat pinnae demonstrated deep inflammation, either alone in 3/9 pinnae or in addition to epidermal inflammation in 6/9 pinnae. Deep perivascular dermatitis and panniculitis was seen in four cases and nodular and/or diffuse dermatitis and panniculitis in five cases. Deep inflammation was also combined with a multi-focal to diffuse perichondritis composed of either histiocytes (5/9), neutrophils (1/9) or mixed inflammatory cells (3/9) (table 2). The auricular cartilage showed focal to diffuse areas of necrosis in all bats and cartilage fragmentation was seen in 8/10 pinnae. Case 3 had haemorrhage, skin and cartilage damage most consistent with trauma during the biopsy procedure. Case 8 had multiple cartilage fragments, tissue remodelling and lack of inflammation suggesting old trauma. Two of five bats (case 4 and 5) with nodular and/or diffuse dermatitis had granulomas located in the mid-to-deep dermis (figure 1C). Vasculitis was identified in one case (case 9, figure 1D). Focal to widespread epidermal necrosis was present in all cases and occurred commonly with crusts in 9/10 cases and ulcers in 7/10 cases. Hyperkeratosis, hyperplasia and spongiosis of the epidermis were also common features and associated with pigmentary incontinence in 7/10 cases (table 2). Microbes were identified in all lesional pinnae and were predominately located in crusts, keratin layers or on the surface of necrotic tissue (table 3). Cocci were seen in all but one case and they were present within epidermal pustules, compatible with a diagnosis of pyoderma, in three (case 2,3 and 6)(figure 2A); bacterial culture yielded staphylococci in two of these (case 2 and 3) but results were not available for the third. Rods were identified in three cases. Bacterial culture results were available from five cases (case 2,3,4,7 and 9), but results only matched morphological identification from H&E slides in three (case 2,3 and 7). On one pinna (case 4), cocci were seen despite a negative bacterial culture, while streptococci and staphylococci were cultured from another pinna (case 9), although cocci were not seen on histopathology. Hyphae or fungal spores were identified on the surface of 8/10 pinnae (H&E slides), including the two cases for which PAS was available (case 2 and 3). In one bat (case 5), fungal hyphae were identified within a granuloma, giving a reasonable diagnosis of fungal infection (figure 2B); fungal culture results were not available for this case. Budding yeasts consistent with *Malassezia* (figure 2C) were found on one pinna (case 10) with marked epidermal hyperplasia and spongiosis. Two fungal cultures were reported as negative, even though hyphae could be seen histologically in surface crusts (case 2 and 3). Findings from special stains (available for eight bats) did not expand those from H&E slides.  Features suggestive of a viral cytopathic effect were noted in pinnal specimens from five bats (cases 2,3,5,6 and 8), where keratinocytes showed enlarged vesicular nuclei or enlarged cytoplasmic vacuoles, perinuclear halos and pyknotic nuclei characteristic of koilocytes14 (table 2, figure 2D). Neoplastic changes were not identified in any pinnal specimens. Both non-lesional reference samples showed pigmented stratified squamous epithelium, dermis (thickness range 10-63um from ten measurements, presumed mid-pinnal), adnexa (simple hair follicles adjacent to large foamy sebaceous glands), prominent neurovascular triad, thin subcutaneous layer, striated muscle, thick perichondrium comprising two layers (outer fibrous layer and inner chondrogenic layer) and centrally located auricular cartilage. Adipose tissue was prominent at the rounded (distal) edge of the pinnal specimen and sparsely distributed along the rest of the length of auricular cartilage.  |
| **DIFFERENTIAL DIAGNOSIS** |
| Differential diagnoses for pinnal lesions in bats included infectious agents (bacteria, fungi, viruses, parasites), immune-mediated disease (auricular chondritis, primary vasculitis), frost-bite, neoplasia and trauma from accident, predation or insect bites.  |

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| **TREATMENT *If relevant***  |
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| **OUTCOME AND FOLLOW-UP**  |
| Clinical information relating to the ten bats was often incomplete (table 1). However, resolution of pinnal lesions was reported in one bat (case 10) after two weeks of oral (enrofloxacin) antibacterial therapy. Response to oral (amoxicillin/clavulanate) and topical antibacterial therapy was equivocal in the group of five bats (cases 2-6) from Kapiti Island, where extensive tissue necrosis ultimately resulted in pinnal amputation, reduced agility of flight and return of affected bats to captivity.10,15 It is not known whether the hair loss experienced by two of the Kapiti Island bats15 (Table 1) resolved with antibiotic therapy. Another bat (case 8) had pinnal lesions at the time of death from dilated cardiomyopathy, despite treatment with topical therapy six months ante mortem. One bat (case 9) was euthanised due to rapidly advancing pinnal necrosis. In the two cases biopsied post-mortem, it is unclear whether additional skin lesions were present at the time of death, however this is considered unlikely given the lack of samples collected from non-pinnal sites. The clinical outcome of two bats, alive at the time of pinnal biopsy, (cases 1 and 7) is unknown.  |

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| **DISCUSSION** |
|  No single aetiological agent could be found as the primary cause of pinnal disease in bats in this case series. Although pyoderma was confirmed in two bats, primary triggers for infection remained elusive. In mice, epi-cutaneous inoculation of pinnae with *Staphylococcus aureus* has been shown to induce epidermal necrosis and permanent loss of pinnal tissue16 and staphylococcal exfoliative toxins have also been proposed as the cause of ear necrosis syndrome in pigs.17 Although *S. aureus* was the predominant pathogen in a study on captive Livingstone’s fruit bats,18 *S. aureus* was not isolated from any of the five samples submitted for bacterial culture. Contagious skin disease is likely where multiple in-contact bats show similar signs of pinnal disease. An unknown infectious agent was proposed as the primary cause of pinnal disease in the group of 20 *Mystacina tuberculata* bats translocated to Kapiti Island in 2005.10,15 In addition, White Nose Syndrome caused by the infectious fungus *Pseudogymnoascus* *destructans* is known to infect the skin of the muzzle, wings and pinnae of bats and has resulted in mass mortalities in North American hibernating bats.11 However, in the one bat where histopathology was consistent with fungal infection, a diagnosis of White Nose Syndrome was considered unlikely due to the absence of key histopathological features which include cup-like epidermal erosion, hyphal invasion of hair follicles and sebaceous glands and curved conidia amongst others.11 Microbial overgrowth is often a secondary complication in compromised skin19 and the role of microbial overgrowth in this group of bats remains unclear. Despite microbial overgrowth being seen in the majority of pinnal specimens examined, it was likely secondary in all cases as neither of the known virulent microbes (*S. aureus, P. destructans*) was identified. Given the potential for clinical improvement or resolution of microbial infection/overgrowth and limited options for diagnostic testing in wild bats, empirical therapy may be warranted, at least when clinical signs compatible with infection are severe. Viruses, including papillomavirus and pox virus, have been isolated from bats and associated with skin lesions.20-22 While inclusion bodies suggestive of pox virus were not seen, koilocytes, which may be indicators of papillomavirus infection, were likely in some cases. As only slides and not tissue blocks were available, immunohistochemistry to confirm viral infection could not be performed. However intracellular oedema initiated through other causes may mimic viral cytopathic effects and also needs to be considered. Since three bats had pinnal lesions sampled during winter, frost-bite remained a valid differential diagnosis for those. Frost-bite was proposed as the cause of pinnal loss (or square-eared anomaly) in 56 of 5,863 free-ranging *Myotis lucifugus* bats in North America7 but histopathological findings were largely inconsistent with such a diagnosis in this case series. In addition to frost-bite, trauma from accident or predation could explain fragmentation of auricular cartilage seen in the majority of cases. However, disintegration of auricular cartilage may also occur secondary to perichondritis and cartilage necrosis in lesional pinnae. Although traumatic injury is the most common cause of perichondritis in people,23 surface trauma from insect bites or scratches has also been known to trigger perichondritis, suggesting that inflammation of deep pinnal tissue can occur in the absence of direct trauma to cartilage and potentially as a result of a compromised skin barrier and infection.23 In summary, epidermal changes were frequent and mostly related to microbial infections, while the deep pathology found in almost all specimens highlights the vulnerability of these vital structures. Further analysis of pinnal specimens, including immunohistochemistry and molecular techniques to characterise potential pathogens, may provide key information. Although availability of pathological samples from bats is limited, sharing of resources, as in this study, will help to progress knowledge of skin diseases in bats. Furthermore, early identification, treatmentand prevention of contagious pinnal disease in bats may be critical to achieving success in bat conservation projects.   |
| **LEARNING POINTS/TAKE HOME MESSAGES** |
| * Lesions of the bat pinna can be significant and warrant clinical investigation in rehabilitating bats.
* Deep inflammation, perichondritis and cartilage necrosis were common features in lesional bat pinnae, emphasising their delicate nature.
* No evidence was found for a single aetiological agent as a primary cause of pinnal disease, but microbial complications were common.
* Frequent secondary microbial overgrowth/infection suggests that antimicrobial therapy may be warranted.
* Further investigations and more extensive sampling, including that of bat casualties, will advance our understanding of pinnal lesions in bats and the aetiology of bat skin disease.

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| **FIGURE/VIDEO CAPTIONS** |
|  **Figure 1.** Composite showing gross morphology and key histopathological findings in lesional bat pinnae. **A*.*** *Mystacina tuberculata* bat from Kapiti Island with necrosis of both pinnae*.* Image courtesy of Brett Gartrell. **B.** Intraepidermal pustular dermatitis, deep perivascular dermatitis and panniculitis patterns of inflammation in pinnal tissue from a *Eptesicus serotinus* bat (case 1). Intraepidermal pustule (asterisk). H&E,40x. **C.** Nodular and diffuse dermatitis and panniculitis patterns of inflammation in pinnal tissue from a *Mystacina tuberculata* bat (case 5). Granuloma (asterisk). H&E,20x **D.** Vasculitis pattern of inflammation in pinnal tissue from a *Macroderma gigas* bat (case 9). H&E,400x. **Figure 2.** Composite showing potential aetiological agents in lesional bat pinnae. **A.** Cocci (arrow) within an erupting sub-corneal pustule in pinnal tissue from a *Mystacina tuberculata* bat (case 2). H&E,400x. **B.** Fungal hyphae (arrow) within a granuloma in pinnal tissue from a *Mystacina tuberculata* bat (case 5). H&E,400x. **C.** Yeasts (arrow) within stratum corneum in pinnal tissue from a *Pteropus livingstonii* bat (case 10). Gram,1000x. **D.** Keratinocytes with large irregular or pyknotic nuclei (likely koilocytes) (arrow); prominent keratohyaline granules (asterisk) in stratum granulosum in pinnal tissue from a *Pteropus livingstonii* bat (case 8). H&E,200x. |
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