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Short communication

The cat flea (*Ctenocephalides f. felis*) is the dominant flea on domestic dogs and cats in Australian veterinary practices

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ABSTRACT

This study was undertaken to determine the flea diversity on urban dogs and cats in Australia in 2009-2010. A total of 2530 fleas were recovered from 291 animals (151 dogs, 69 cats and 71 uncategorised dogs or cats) from veterinary clinics across five states of Australia. The majority of specimens were from coastal areas. The cat flea (*Ctenocephalides felis felis*) was the most frequent flea species identified (98.8%, 2500/2530). The only other flea species identified was the stickfast flea (*Echidnophaga gallinacea*) from Western Australia. Sequencing of the cytochrome oxidase subunit II mtDNA revealed a single haplotype across Australia within a subset of *C. f. felis* ($n = 19$). Our study demonstrated dominance and haplotype homogeneity of *C. f. felis* on dogs and cats. Although *Ctenocephalides canis* was recovered from a feral fox, it was not identified from the sample of fleas analysed. This suggests that, under current conditions, it is unlikely that foxes are reservoirs of *C. canis* for domestic dogs or cats residing in coastal Australia, as previously speculated.

1. Introduction

Fleas (Siphonaptera, Pulicidae) are the most important ectoparasites on domestic dogs and cats worldwide (Blagburn and Dryden, 2009). Besides causing discomfort to pets and their owners through biting, fleas cause flea allergy dermatitis (FAD) and are vectors for human *Bartonella* and *Rickettsia* species which can cause disease in both man and animals (Schloderer et al., 2006; Barrs et al., 2010; Bitam et al., 2010; McElroy et al., 2010). Since the late 1980s, modern anti-flea products have revolutionised the way of controlling flea infestation and the associated FAD (Cadiergues et al., 2001; Rust, 2005; Franc and Yao, 2007). Despite the availability of these modern products, flea infestation remains extremely common worldwide.

It is generally assumed that the cat flea (*Ctenocephalides felis*) is the dominant flea causing flea infestations of domestic dogs and cats in Australia (Seddon, 1951a, 1967; Chin et al., 2005; Barrs et al., 2010). However, Schloderer et al. (2006) found four flea species in a survey of domestic dogs and cats. In this study they suggested that *C. felis* was likely to be the most important vector of *Rickettsia felis* in Western Australia, despite also identifying this organism in a stickfast flea (*Echidnophaga gallinacea*) (Schloderer et al., 2006). Although the importance of flea borne diseases is increasing, no recent Australia-wide flea survey has been conducted (Rust, 2005; Barrs et al., 2010).

The aims of this study were to determine the species of flea infesting cats and dogs attending veterinary practices, to provide an updated perspective on flea diversity in households across Australia during the 2009–2010 flea seasons, and to consider past opinion regarding the impact of geography on the prevalence of flea species. To investigate genetic identity we used PCR amplification and sequenc-

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ing of the cytochrome oxidase subunit II gene (*cox2*) to determine the homogenetity of *C. felis* across Australia.

2. Materials and methods

2.1. Flea specimens

Two cohorts of fleas were collected for this study. The first (1) cohort of fleas was collected, together with a basic history, from random individual dogs and cats presented to veterinary clinics. All fleas were collected between November 2009 and April 2010 (summer/autumn in Australia). The second (2) cohort of fleas was obtained prospectively through a diagnostic survey designed to obtain current information on the flea species infesting cats and dogs (circulated in the Control & Therapy Series – 256 December 2009; Centre for Veterinary Education; http://www.cve.edu.au/candt). All fleas were collected between January 2010 and August 2010 (summer/autumn/winter in Australia).

Fleas were collected by veterinarians and placed in 70–100% ethanol. Flea containers were labelled with the postal code of the veterinary clinic. Fleas were from an individual animal (cohort 1, some in cohort 2) or a number of animals (cohort 2). All unsorted/unidentified fleas were shipped to the University of Sydney for sorting and species determination.

Fleas on a feral red fox (*Vulpes vulpes*) were collected from Inglewood, Queensland in 2007 and kindly donated for identification by Dr Graeme Brown (University of Sydney). Fleas from a short beaked echidna (*Tachyglossus aculeatus*) from Lithgow, New South Wales were submitted to the Faculty of Veterinary Science, University of Sydney in October 2010 for identification.

Coordinates of all flea sampling locations were plotted on a map of Australia with the aid of Google Maps (Google).

2.2. Morphological identification of fleas

Fleas were examined using a stereo-microscope (5–20× objectives, Olympus). The species and sex of the flea was recorded. Voucher specimens were cleared in 10% potassium hydroxide, dehydrated through an ethanol series, transferred to xylene, slide-mounted in DPX Mount for histology (44581, Sigma), and then examined under a BX41 Olympus microscope. Fleas were identified using descriptions and/or keys provided in detailed taxonomical overviews by Dunnet and Mardon (1974) and Hopkins and Rothschild (1953). Dunnet and Mardon (1974) provide a comprehensive historical account of all Australian voucher specimens of fleas recorded up to the 1970s, including those found on domestic dogs and cats.

2.3. Flea genotyping using cytochrome oxidase subunit II gene sequence

Total DNA was extracted from fleas using an ISOLATE Genomic DNA Mini Kit (Bioline, Australia), with the following initial modification. Briefly, a whole flea or a flea leg (to preserve the morphological specimen for mounting) was placed in a 1.5 mL tube in 400 μL of lysis buffer with Proteinase K and was ground for 3 min using 425–600 μm diameter glass beads (G8772, Sigma–Aldrich, Australia) and a plastic pestle (Eppendorf, Australia). DNA was eluted into 200 μL of elution buffer. The cytochrome oxidase subunit II gene sequence from the mtDNA (*cox2*) was amplified using F-Leu (5′ TCT AAT ATG GCA GAT TAG TGC′ 3) and R-Lys (5′ GAG ACC AGT ACT TGC TTT CAG TCA TC′ 3) according to Whiting et al. (2008). We used Econo*Taq* PLUS GREEN MasterMix (Lucigen, USA), KAPA2G™ Robust HotStart ReadyMix, KAPA2G™ Fast HotStart ReadyMix (KAPA BioSystems, MA) or MyTaq™ HS Mix (BioLine, Australia). Primers were added at 0.25 μM concentration. The PCR cycle was adjusted according to the master mix used, annealing temperature was set to 42 °C and we employed 37 cycles (Brinkerhoff et al., 2011). The PCR was performed in an Eppendorf Mastercycler Personal. Resulting amplicons were resolved using 1.5% (w/v) agarose gel with GelRed™ Nucleic Acid Gel Stain (Biotium, USA) in 1X TBE buffer. All PCRs contained a negative control consisting of sterile water. PCR products were sequenced at the 1st BASE (Singapore) or the Australian Genome Research Facility (Australia). Sequences were assembled, aligned with related sequences and analysed using the CLC Main Workbench 5.6 (CLC bio, Denmark). Sequences obtained in this study were submitted to GenBank™ (**HQ696926-HQ696946**).

3. Results

A total of 2530 fleas belonging to two species were recovered from 291 cats and dogs (151 dogs, 69 cats and 71 uncategorised dogs or cats) from five states of Australia (Fig. 1 A, Tables 1–3). The cat flea (*C. f. felis*) was the most frequent flea species identified ($n = 2500$) throughout Australia. The only other flea species identified was the stickfast flea (*E. gallinacea*, $n = 30$), all from Western Australia. For *C. f. felis*, there was a preponderance of females (1892) over males (581), sex ratio 3.4 (1892/581; Tables 1–3). Similarly for *E. gallinacea*, there were 25 females and only 5 male fleas (sex ratio 5, 25/5; Table 3).

The fleas from cohort 2 were predominantly from greater Sydney region with 135 out of 150 animals from New South Wales with 24 dogs, 33 cats and 78 unrecorded dogs or cats within 40 km from Sydney CBD. Only a few collection sites and animals were from other states (Fig. 1A, Tables 1–3). The majority of fleas were from coastal areas (Fig. 1A). Fleas from outside coastal areas were from central New South Wales (Wagga Wagga and Walgett, $n = 13$, all *C. f. felis*) and from the Blue Mountains, New South Wales (Katoomba, $n = 20$, all *C. f. felis*) (Fig. 1 A). The fleas on the feral fox were dog fleas (*C. canis*, $n = 7$) and *C. f. felis* ($n = 4$). The fleas on the echidna were *Bradiopsylla echidnae* ($n = 37$).

Morphologically identified *C. f. felis* from around Australia were randomly selected for genotyping using *cox2* (Fig. 1 B). All PCR reactions yielded ~770 bp amplicon spanning the complete *cox2* (684 bp, 228 aa). All *C. f. felis* haplotypes were 100% identical ($n = 17$). During our morphological identification, two fleas from greater Sydney, New South Wales (Belmont, Rooty Hills) exhibited variable morphology of one of their hind tibia, i.e. two notches