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Seroprevalence of *Coxiella burnetii* in domesticated and feral cats in eastern Australia

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ABSTRACT

The seroprevalence of *Coxiella burnetii* (*C. burnetii*) in cats in eastern Australia is unknown, and the risk of transmission from cats to humans is undetermined. This study aimed to determine the exposure of cats to *C. burnetii* in four distinct cat subpopulations. An indirect immunofluoresence assay (IFA) and an Enzyme-linked immunosorbent assay (ELISA) used for detection of anti-*C. burnetii* antibodies in humans were adapted, verified for use on feline serum, and compared. Cat serum samples ($n = 712$) were tested with IFA from four subpopulations [cattery-confined breeding cats, pet cats, feral cats and shelter cats]. The proportions of seropositive cats were; cattery-confined breeding cats (35/376, 9.3%), pets (2/198, 1%), feral cats (0/50), shelter cats (0/88). The significant variables in *C. burnetii* seropositivity were cattery-confined breeding cat subpopulation and sterilisation status, with infected cats 17.1 (CI 4.2–70.2; $P < 0.001$) times more likely to be cattery-confined breeding cats and 6.00 (CI 2.13–16.89; $P < 0.001$) times more likely to be entire than sterilised. ELISA was used on 143 of 712 sera tested with IFA, and the Cohen's Kappa coefficient of 0.75 indicated 92.2% agreement between the two assays. These results confirm that Australian cats have been exposed to *C. burnetii* and that a higher seroprevalence of *C. burnetii* is seen amongst cattery-confined breeding cats. Cat breeders and veterinary personnel involved in feline reproductive procedures may be at higher risk of exposure to *C. burnetii*.

1. Introduction

Q fever is an important worldwide zoonosis caused by the bacterium *Coxiella burnetii*. For the 40% of primary infections that are symptomatic and clinically polymorphic in humans, illness can be acute or chronic with the potential for serious and debilitating sequelae such as endocarditis, post-Q fever fatigue syndrome and recrudescent granulomatous lesions in bone or soft tissue (Babudieri, 1959; Marrie, 1990).

The causative bacterium has a potentially large, seemingly asymptomatic reservoir encompassing wild and domestic mammals, birds and arthropods (Babudieri, 1959; Baca and Paretsky, 1983). It has two diverse aspects to its lifecycle; a metabolically active form that obligately replicates within the monocyte/macrophage cell lineage and an inactive but environmentally resilient form that travels to new hosts. *C. burnetii* localises in the uterus and mammary glands of mammals with products of conception containing the highest concentration of organisms (Babudieri, 1959; Welsh et al., 1958). Humans acquire infection from animals directly or indirectly mainly via inhalation of contaminated aerosols (Babudieri, 1959; Maurin and Raoult, 1999). Infected domestic ruminants

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including cattle, sheep and goats are the most commonly reported sources of infection for humans however a broader view on the potential of other animal reservoirs to impact on public health worldwide has developed more recently (Angelakis and Raoult, 2010; Woldehiwet, 2004).

Diagnosing an infection with *C. burnetii* in humans primarily relies on serological tests due to their higher sensitivity compared with nucleic acid amplification on blood and tissue samples which is often reserved for acute infections. The presence of antibodies to *C. burnetii* provides evidence of recent or past exposure to the bacteria. The three main serological tests used in human medicine are complement fixation test (CFT), indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) (Fournier et al., 1998). In animals, CFT, which has many limitations, is the only validated serological test available (Rousset et al., 2010). It is the least sensitive method, regularly failing to detect antibodies in sheep and goats, has difficulty diagnosing recent infections (Arricau-Bouvery and Rodolakis, 2005) and is inferior to both IFA and ELISA in humans (Cowley et al., 1992; Peter et al., 1985) which can evaluate class-specific IgG, IgM and IgA antibodies (Peter et al., 1988). In the CFT used for animals, class-specific immunoglobulins are simultaneously tested, but the end titre does not reflect which immunoglobulin was detected.

A recent outbreak of Q fever in veterinary personnel in a Sydney small animal veterinary hospital following a caesarean section in a healthy queen highlighted the potential role domestic cats may play as reservoirs of infection (Kopecny et al., 2013; Maywood, 2011). Establishing the seroprevalence of *C. burnetii* in different subpopulations of cats is essential in order to obtain an overview of recent or past exposure of cats to this bacterium to determine the risk cats may pose to veterinary personnel, professional cat breeders and pet owners and to gauge the likelihood of it being an agent of disease in cats. Seroprevalence studies in cat populations report great variability in the prevalence of *C. burnetii* in different geographical locations and time periods. Additionally, the methodology used has been highly variable even when the same assay type is used. This study aimed to (1) develop a serological method for detecting antibodies to *C. burnetii* in domestic cats (*Felis domesticus*) by adaptation and optimisation of commercially available IFA and ELISA kits developed for use on human serum and (2) determine the seroprevalence of *C. burnetii* in a variety of cat subpopulations to determine the potential risk to population health using a retrospective observational study.

2. Materials and methods

2.1. Sample population

Convenience sampling resulted in the categorisation of cats into four distinct cat subpopulations; (a) pet cats ($n = 198$); (b) cattery-confined breeding cats ($n = 376$); (c) cats entering, or housed in, animal shelters or council pounds ($n = 88$) and (d) feral cats ($n = 50$). All cattery-confined breeding cats were located in eastern Australia, predominantly in New South Wales (NSW) ($n = 292$) with remaining cats from Victoria (VIC) ($n = 26$), Tasmania (TAS) ($n = 12$) and Queensland (QLD) ($n = 46$). Information regarding breed, age, gender (including neuter status), physical findings, medical history and housing type (exclusively indoors, exclusively outdoors or combination) was obtained from medical records (pet cats) or cattery owners (cattery-confined breeding cats). For feral cats, the gender, estimated age and abnormal physical findings were recorded.

A separate group of cattery-confined breeding cats from a single cattery ($n = 27$) including the index cat at the centre of the Q fever outbreak at a small animal hospital in Sydney (Kopecny et al., 2013) and 26 cats residing within the same cattery were used as an essential part of the optimisation process but excluded from calculations of seroprevalence.

The serum samples from pet cats ($n = 198$) were archived samples used in previous studies (Bell et al., 2006; Norris et al., 2007), collected when cats presented to veterinary clinics [University Veterinary Teaching Hospital, Sydney (UVTH-S), Paddington Cat Hospital (PCH) and Concord Animal Hospital]. The archived samples comprised residual serum taken from cats during routine veterinary services for disease investigation or health checks.

Serum samples from 376 cattery-confined breeding cats were tested. Of these, 345 were from samples previously used in a FIV prevalence study (Norris et al., 2007) from catteries in the greater Sydney region. Permission to repeat sample collections from certain cats ($n = 7$) was granted by cattery owners. Simultaneously, samples from new cats ($n = 24$) at these catteries were collected and an additional subset of samples from a cattery not previously sampled was collected ($n = 7$). Therefore, the total number of cattery-confined cats sampled was 376 with 345 cat sera from archived studies and 31 sera collected following initial screening.

The shelter cat subpopulation consisted of cats entering or housed within animal shelters and council pounds ($n = 88$). Forty-four of these serum samples came from cats older than 18 months of age, either housed or entering specific animal shelters in NSW that were part of another study investigating feline heartworm prevalence (Animal ethics approval number 5072.04-11 SWAHS AEC) and were donated for the current study ($n = 44$). The remaining 44 samples were from cats presenting as strays to a council facility in south-western Sydney.

The feral cat population ($n = 50$) comprised two separate colonies forming part of a FIV prevalence study (Norris et al., 2007). Samples had been collected from one colony of feral cats residing near a piggery in Menangle in south-western Sydney and the second colony of feral cats living around the University of Sydney's Camperdown campus was the focus of a 'neuter and release' campaign.

2.2. Sample collection

Whole blood (0.5–3 mL) from each cat was collected into serum separator tubes (BD Vacutainer SST II, Sydney, Australia) and centrifuged at $8000 \times g$ for 10 min. Serum was harvested and stored in 300 μL aliquots at −20 °C until analysed.