A SEROSURVEY OF CATS FOR RICKETTSIA ANTIBODIES IN EASTERN TEXAS

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ABSTRACT

Background. *Rickettsia* sp. bacteria are arthropod borne and a number of them are capable of causing human infections. A number of studies have indicated that domestic cats and dogs may potentially serve as reservoirs of several *Rickettsia* species and may contribute to human infections. The purpose of this study was to determine if cats in eastern Texas exhibited indications of rickettsial infection that could indicate a potential source of human exposure.

Methods and Findings. Serum collected from 188 cats across 3 counties in eastern Texas was subjected to ELISA assays to determine if antibodies to *Rickettsia typhi* were present. Overall, 30% of cats were seropositive for the *R. typhi* antigen used.

Conclusions. In the eastern Texas area a significant percentage of cats are seropositive for *R. typhi* and may represent a potential reservoir and source of human infections.

Keywords: Rickettsia, zoonosis, ticks.

1. INTRODUCTION

Members of the bacterial family Rickettsiaceae are gram negative, obligate intracellular parasites. Of the three genera in the family, *Rickettsia*, *Orientia*, and *Wolbachia*, most human pathogens are contained within the *Rickettsia* genus. *Rickettsia sp.* are typically maintained with a host / vector system in which the bacteria are passed among vertebrate hosts by arthropod vectors (1). Humans become infected when they are exposed to an infected arthropod vector.

Traditionally, pathogenic *Rickettsia sp.* have been divided into either the Spotted Fever Group (SFG) or the Typhus Group (TG). While human infections produced by the groups are almost clinically identical, SFG and TG rickettsia may be distinguished based on outer membrane proteins and their vectors – SFG are typically transmitted by ticks while TG are transmitted by fleas and lice (2). In the United States, Rocky Mountain Spotted Fever (causal organism, *Rickettsia rickettsia*) has been a reportable illness since 1944 (3). Beginning in 2010, the reportable condition was changed to Spotted Fever Rickettsiosis (SFR) and expanded to include all cases of confirmed or suspected rickettsial infections. In 2013, 3181 suspected cases and 174 confirmed cases of SFR were reported; this included 83 cases in Texas (4).

Previous work by this author found that, in an east Texas population, almost 16% of individuals were seropositive for *Rickettsia typhi* or *Rickettsia felis* antibodies (5). While these bacteria are classified as TG (*R. typhi*) and SFG (*R. felis*), they are, in the United States, maintained by the same arthropod vector / vertebrate host system: *Ctenocephlides felis* (cat flea) and *Didelphis virginiana* (opossum) (6,7,8) and infections with either are not clinically distinguishable from one another (2). The cat flea is a widely spread ectoparasite of both cats and dogs; in fact, *C. felis* is believed to be the most common flea on cats and dogs (9). It is also clear that both dogs and cats can become infected with *R. typhi* and *R. felis* (10,11,12,13). The goal of this study was to determine if cats in the east Texas region exhibited serologic evidence of *R. typhi* or

R. felis infections and whether they might serve as rickettsia reservoirs and contribute to human infections.

2. MATERIALS AND METHODS

Cat serum. Veterinarians in three east Texas counties, Smith, Nacogdoches, and Angelina, were contacted and agreed to obtain cat serum, collected as part of normal medical procedures, for this project. For each cat the following information was noted at the time of serum collection: gender, approximate age, and what environment the cat lives in (i.e. indoors, indoors/outdoors, and outdoors). The veterinarians collected the serum in Vacutainer SST serum separation tubes (Becton Dickinson), centrifuged it for separation, and stored the samples at 4 C until delivered to our laboratory, whereupon 1.5 ml of serum was frozen at -70°C until analysis. A total of 188 cat serum samples were collected: 36 from Angelina county, 124 from Nacogdoches county, and 38 from Smith county.

Enzyme–linked immunosorbent assays (ELISA's). *R. typhi* (Wilmington strain cultivated in chicken embryo yolk sacs) was diluted 1:4000 phosphate buffered saline (138 mM NaCl, 10 mM PO4, 2.7 mM, pH 7.4) with 0.1% Tween 20 and 1% bovine serum albumin (BSA). Four wells in the ELISA plate received PBS only – these served as negative controls. Prior to the application of cat serum, the ELISA plates were rinsed with large amounts of PBS. Cat serum samples were diluted 1:2000 in PBS and 100 µl of the dilution was added to all ELISA plate wells except the four negative control wells – these received PBS only. ELISA plates were then incubated for 30 minutes at 37°C. After several rinses with PBS, a 100 µl of a 1:250 dilution of goat anti–cat IgG-Fc horseradish peroxidase conjugated antibody (Bethyl Laboratories, Montgomery, TX) was applied to all wells and incubated for 30 minutes at 37°C. After rinsing with large amounts of PBS, 100 µl of a colorimetric detection solution (0.8% ABTS and 0.2% hydrogen peroxide) was applied to each well and the plate again incubated for 30 minutes at 37°C. Colorimetric changes were quantified by reading the absorbance of each well in an

Anthos microplate reader at 405 nm. To be considered positive for the presence of *R. typhi* antibodies, a serum sample had to produce a mean absorbance, at 405 nm, of greater than the mean absorbance of the four negative control wells plus three standard deviations.

Data analysis. Chi-square testing was performed upon seropositive cats to look for associations with age, county of residence, or environment.

3. RESULTS AND DISCUSSION

Overall, out of 188 serum samples collected, 56, or 29.8%, were positive for *R. typhi* antibodies. By county, Angelina had 10 seropositive cats (28%), Nacogdoches had 30 seropositive cats (24%) while Smith had 16 seropositive cats (33%). Chi-square testing indicated no significant difference between seropositive rates and the 3 counties (χ^2 1.85, p = .67). With no county differences, all cats were considered as a single large sample and further chi-square testing was conducted based on the data collected by the veterinarians. Table 1 summarizes data regarding age, gender, and environment versus seropositive status. Persons Chi-square testing revealed no significant differences regarding seropositive rates and age (χ^2 1.17, p = 0.277, gender (χ^2 = 2.867, p = .412), or environment (χ^2 = 1.003, p = 0.61).

Recent studies have found that domestic dogs and cats may serve as reservoirs, and contribute to the spread of, both TG and SFG rickettsia. In Spain, 12% of surveyed dogs were seropositive for *R. typhi* (11) while 44% of surveyed cats were seropositive for *R. conorii* (14). In another study from Spain, 35% of cat sera were seropositive for *R. typhi* and 55% of cat fleas collected were positive for *R. typhi* DNA as assayed via PCR (10). This study collected some of the same demographic data on the surveyed cats as we did and also found no correlation to age, environment, or even ectoparasite infestation at the time of sampling. While this study and most others have focused on cats, a survey from Australia found that dogs were positive for *R. felis* DNA (13).

A number of studies have looked at the possibility that cats might serve as

reservoirs for rickettsial pathogens (7,15,16) and a number of recent papers have detailed the detection of rickettsial infection in cats. In one Spanish study, over 16% of cats were seropositive to *R. felis* (17) while in a second, cats were seropositive for three different rickettsial pathogens: *R. massiliea*, *R. conorii*, and *R. felis* (18). A study conducted in Bangladesh found evidence that cats played a role in the transmission of *R. felis*, noting the infection of fleas, the cats they were collected from, and patients with febrile illnesses were correlated (12).

Previous research by this author and others has found that while 16% of individuals from the east Texas region were seropositive for the *R. typhi* antigen used in this study (5), we found no correlation to seropositive status and pet ownership (dogs or cats). However, based upon the growing body of evidence described above and the data from this current study, it appears that domestic cats may serve as potential reservoirs for rickettsial infection and that flea control could serve to reduce the risks of human exposure. It would also be wise for doctors, when confronted with a patient suffering from a febrile illness resembling a rickettsial infecton, to at least inquire as to whether or not a cat resides in the household and take this into account during diagnosis and treatment.

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Table 1. Summary of data associated with cat serum samples

	Male	Female	<1 year	1 – 5 years	>5 years	Indoor	Indoor / outdoor	Outdoor
Number of cats	**106**	**82**	**65**	**90**	**33**	**26**	**149**	**13**
# seropositive	**34**	**22**	**15**	**29**	**12**	**6**	**47**	**3**
% seropositive	**32**	**27**	**23**	**32**	**36**	**23**	**32**	**23**

