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Acquisition of *Rickettsia felis* by Cat Fleas During Feeding

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Abstract

Evidence for horizontal routes of transmission for *Rickettsia felis* has come from detection of *R. felis* infection in vertebrates and multiple blood-feeding arthropods; however, infection of cat fleas, *Ctenocephalides felis*, during blood feeding has not been demonstrated. In this study, the ability of cat fleas to acquire *R. felis* through an infectious blood meal with subsequent vertical transmission was examined. Utilizing an artificial feeding system, *Rickettsia*-naive fleas were exposed to *R. felis*–infected blood meals and monitored for subsequent infection at weekly intervals for 4 weeks. At 7 days postexposure (dpe) ~52% of fleas successfully acquired rickettsiae and *R. felis* DNA; rickettsial transcript and DNA was detected in cat flea feces. Quantitative real-time polymerase chain reaction determined that both the *R. felis* infection load and *R. felis* infection density was significantly greater in fleas assessed at later time points. Although a persistent *R. felis* infection was detected in adult fleas, *R. felis* infection was not observed in F_1 progeny. This study demonstrates that cat fleas are able to acquire *R. felis* infection from an infectious blood meal and will serve as a model to examine *R. felis* transmission between arthropod and vertebrate hosts.

Key Words: Cat flea—Flea-borne disease—*Rickettsia felis*.

Introduction

Rickettsia felis, a gram-negative bacterium, has been identified from both vertebrates and arthropods worldwide. In humans, *R. felis* is considered an emerging pathogen as the etiologic agent of flea-borne rickettsiosis (Perez-Osorio et al. 2008). Clinical characteristics of *R. felis* rickettsiosis range from a mild to moderate flu-like illness with symptoms similar to other rickettsial infections, including fever, headache, and fatigue to severe disease associated with hepatic, pulmonary, and central nervous system manifestations (Zavala-Velazquez et al. 2000, Zavala-Castro et al. 2009). Molecular and serological surveys have detected *R. felis* infection of peridomestic mammals that commonly serve as hosts for fleas, including dogs, cats, and opossums (Schriefer et al. 1994, Richter et al. 2002, Labruna et al. 2007); however, a persistently infected vertebrate reservoir has yet to be clearly implicated in the transmission cycle.

Since the first description as the ELB (Elward Lab) agent in a commercial cat flea (*Ctenocephalides felis*) colony in 1990 (Adams et al. 1990), *R. felis* has been associated with at least 22 arthropod species worldwide (Berrelha et al. 2009, Blair et al. 2004, Maioli et al. 2009, Reif and Macaluso 2009, Varagnol et al. 2009). Utilization of molecular assays, traditional and quantitative real-time polymerase chain reaction (qPCR), has increased the known flea hosts associated with *R. felis*; however, only *C. felis* is currently recognized as a biological vector. Under laboratory conditions, *R. felis* is predominantly maintained within cat flea cohorts by vertical (transovarial and transstadial) transmission (Azad et al. 1992, Wedincamp and Foil 2002). The capacity to maintain *R. felis* infection via vertical transmission decreased from ~65% to <5% when the colony was exposed to rickettsiae-free blood over 12 generations (Wedincamp and Foil 2002). Such decrease in prevalence in the absence of an infectious blood meal, combined with the increasing diversity of infected fleas, suggests that transmission mechanisms outside the vertical route are necessary for maintenance of *R. felis* in flea populations.

A complex transmission cycle of flea-borne rickettsial diseases has been described previously. *Rickettsia typhi*, the agent of flea-borne murine typhus, is primarily transmitted to vertebrate hosts via infectious flea feces. However, *R. typhi* can be ingested by fleas during blood meal acquisition and transmitted both vertically to progeny and horizontally to cofeeding fleas via the vertebrate host blood (Azad 1990). Evidence for horizontal routes of transmission for *R. felis* has come from detection of *R. felis* infection in vertebrates and multiple blood-feeding arthropods; however, infection of cat

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fleas during blood feeding has not been demonstrated. In the current study, the ability of cat fleas to acquire *R. felis* through an infectious blood meal and subsequently pass the organism to their progeny was examined. Using an artificial flea feeding system, the kinetics of rickettsial infection of *Rickettsia*-naive cat fleas were examined using a qPCR assay. Additionally, vertical transmission of *R. felis* to progeny by newly infected cat fleas was assessed. Although acquisition and persistent infection of cat fleas with *R. felis* was observed, vertical transmission was not evident. Implications of these results in the context of sustained *R. felis* transmission are discussed.

Materials and Methods

Source of fleas and Rickettsia

Newly emerged, unfed cat fleas (*C. felis*) were purchased from Elward II (EL). Cat fleas from EL Laboratory have been previously reported to be specifically free of *R. felis* infection (Pornwiroon et al. 2007). Adult fleas were provided a blood meal via an artificial dog (Wade and Georgi 1988), and eggs, not separated from feces, were reared to adults on sand with artificial diet as previously described (Lawrence and Foil 2002). *R. felis* (LSU), originally isolated from the Louisiana State University cat flea colony, was propagated in an *Ixodes scapularis*–derived cell line (ISE6) maintained in modified L15B growth medium, and cellular infection was examined by Diff-Quik (Dade Behring) staining as described previously by Pornwiroon et al. (2006).

Nucleic acid isolation

Individual sampled fleas were assigned sample numbers, placed in 0.5 mL microcentrifuge tubes, and pulverized with sterile plastic pestles in a liquid nitrogen bath. Genomic DNA (gDNA) was extracted using a modified version of the HotSHOT DNA extraction protocol (Truett et al. 2000). Briefly, individual flea lysates were incubated at 95°C for 45 min in 20 μL alkaline lysis reagent (25 mM NaOH and 0.2 mM disodium ethylenediaminetetraacetic acid, pH 12), cooled to 4°C for 5 min, and mixed with 20 μL of neutralizing reagent (40 mM Tris-HCl, pH of 5). All gDNA preparations were stored at −20°C. For flea feces, at 2, 4, 7, 14, 21, and 28 days postexposure (dpe), ~50 mg of egg-free flea feces were collected and dissolved in 200 μL of 1×phosphate-buffered saline. Extraction of gDNA from feces was accomplished using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions for extraction of DNA from blood samples and eluted in 40 μL Buffer AE. Total RNA from flea feces was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions for total RNA isolation from cells with the following modifications: ~50-mg of feces was disrupted and homogenized with two stainless steel 4.77-mm balls in 200 μL of Buffer RLT using a TissueLyser (Qiagen), for 1 min at 30 Hz. Samples were washed as directed with an additional wash in 500 μL Buffer RPE and eluted in 30 μL RNase-free water. RNA samples were DNaseI treated (Promega) according to the manufacturer's instructions. Gene-specific cDNA was synthesized using SuperScript First-Strand Synthesis System (Invitrogen) with *R. felis* 17-kDa primers (Reif et al. 2008). For all samples no-RT controls were included to verify the absence of DNA contamination.

Rickettsial detection and quantification by PCR

Tissue culture samples or individual fleas were assessed for *R. felis* infection by qPCR amplification of a 157-bp portion of the *R. felis* 17-kDa antigen gene as previously described (Reif et al. 2008). To confirm the absence of rickettsial infection before exposure to *R. felis*, a subset of fleas was additionally screened by qPCR amplification of a 128-bp portion of the rickettsial outer membrane protein B (*ompB*) gene (Labruna et al. 2007). Briefly, qPCR components and the template that included 2×iTaq SYBR Green Supermix (BioRad); 100 nM of each primer; DNase/RNase-free water; and 5 μL of gDNA template (samples), water (negative control), or serial 10-fold dilutions (1×10^8 to 10 copies) of pCR4-TOPO-*Rf*17kDa+*Cf*18SrDNA were premixed in 35 μL volumes in 96-well plates and aliquoted in triplicate 10 μL reactions on 384-well plates. The qPCR was performed with an ABI 7900HT unit (Applied Biosystems) using conditions previously described (Reif et al. 2008). Results were analyzed with ABI 7900HT sequence detection system (SDS v2.3) software. The specificity of the assay was verified; the expected single peak for the internal control plasmid and positive gDNA samples, but not in the water (negative control) samples, was identified in the dissociation curve. Additionally, representative qPCR products from each trial were verified by gel analysis to confirm the specificity of the reaction; cloning and sequencing confirmed that fleas were infected with *R. felis*. The sensitivity of this qPCR is 10 copies of plasmid per reaction or ~267 rickettsiae per 40 μL of flea lysate. For *R. felis*–positive flea samples, a second assay determined *R. felis* infection density in individual fleas. Both the *R. felis* 17-kDa and cat flea 18S rDNA genes were amplified and quantified by extrapolating the individual gene Ct values from serial dilutions of plasmid pCR4-TOPO-*Rf*17kDa+*Cf*18SrDNA, which contained single-copy portions of both genes as previously described (Reif et al. 2008).

R. felis*–infected blood meal preparation*

Exposure blood meals were prepared by resuspending a confluent T-75 flask containing ISE6 cells >90% infected with *R.* felis (passage 4). To prepare the *R. felis* exposure dose, *Bac*Light viability stain kit (Invitrogen) was used to assess viability and enumerate rickettsiae as previously described (Sunyakumthorn et al. 2008). The concentration of rickettsiae in culture was additionally verified by qPCR amplification of the *R. felis* 17-kDa gene. The *R. felis*–infected cell solution was diluted to 5×10^9 rickettsiae, subjected to centrifugation at 13,000 *g* for 10 min to remove the growth medium, and resuspended in 600 μL of heat-inactivated, defibrinated bovine blood (Rockland Immunochemicals). Intact, *R. felis*–uninfected ISE6 cells were prepared in an identical manner and served as a negative control for the feeding treatment.

Acquisition of R. felis *via infectious blood meal*

In two replicate trials, each containing three identical experimental groups and one control group, fleas (125 mixed sex fleas per group) were exposed for 24 h to either a *R. felis*–infected or –uninfected blood meal. After the exposure meal, cat fleas were maintained on defibrinated bovine blood (not heat-inactivated) that was replaced every 1–3 days for the duration of the experiment. Fleas were examined at weekly intervals for acquisition and persistence of *R. felis* infection. At

each collection point (7, 14, 21, and 28 dpe), gDNA was extracted from 10 individual randomly selected fleas and *R. felis* infection was determined by qPCR. For each trial the mean (1) incidence of *R. felis* infection, (2) *R. felis* infection load per flea lysate, and (3) *R. felis* infection density were compared at each time point.

Detection of R. felis *in cat flea feces*

A portion of egg-free flea feces was collected at 2, 4, 7, 14, 21, and 28 dpe from groups of fleas fed either an *R. felis*–infected or –uninfected blood meal. To determine the presence and quantity of *R. felis* gDNA, 50-mg samples of feces were examined by qPCR amplification of a portion of the *R. felis* 17-kDa gene. To assess the viability of *R. felis* in flea feces, qPCR was used to amplify *R. felis* 17-kDa-specific cDNA. Due to limited starting material, rickettsial transcript was only assessed in trial 1 on days 9, 21, and 28 dpe.

Vertical transmission of R. felis *to flea progeny*

In both trials, eggs from fleas exposed to *R. felis*–infected blood meals were collected, pooled by week postparental blood meal exposure, and reared to adults in an incubator maintained at 27°C and 70% relative humidity. From each group, 10 unfed first-generation (F_1) adult cat fleas, collected as eggs 1–4 weeks postparental exposure to the *R. felis*–infected blood meal, were randomly selected and examined for *R. felis* infection. Total gDNA was extracted from F_1 cat fleas and the presence of *R. felis* and sample quality were determined by qPCR, as described above.

Statistical analysis

Rickettsial load in fleas and the ratio of *Rf*17kDa/*Cf*18S were assessed after the logarithmic transformation of the quantity of the genes of interest (*Rf*17kDa and *Cf*18S). Analysis of variance (SAS statistical package, Version 9.1.3, GLM procedure) was performed to examine potential differences between *R. felis* infection load in fleas and ratio of *Rf*17kDa/*Cf*18S copy number over the study period; when overall significance was found, Tukey's honestly significant difference *post hoc* test was used to examine pairwise differences of means of main effects. Pairwise *t*-tests of least squares means were performed to determine any interaction effects between trial, group, and day for *R. felis* infection load and ratio of *Rf*17kDa/*Cf*18S. An *F*-test was used for general comparisons of grouped means. For all comparisons, a *p*-value of <0.05 was considered significantly different.

Results

Acquisition and growth of R. felis *in naive fleas exposed to a* R. felis*–infected blood meal*

To verify the absence of *R. felis* infection in this colony 20 (10 female and 10 male) newly emerged unfed EL fleas were assessed for *R. felis* infection by qPCR amplification as previously described (Reif et al. 2008). No rickettsial product was amplified from any flea sample, confirming the absence of *R. felis* in the EL *C. felis* colony as documented in previous reports (data not shown) (Pornwiroon et al. 2007). In replicate trials, three groups of fleas were fed *R. felis*–infected blood meals and a fourth group (control) was fed an uninfected blood meal for 24 h. After the exposure meal 10 fleas per group were assessed for *R. felis* infection at weekly intervals. The specificity of *R. felis* infection in fleas was confirmed by sequencing a portion of the rickettsial 17-kDa gene from a representative subset of fleas positive for *R. felis* infection. All sequenced samples had a 100% nucleotide identity to *R. felis* URRWXCal2, complete genome (GenBank accession number CP000053).

All groups fed an *R. felis*–infected blood meal had *R. felis*–positive individuals for up to 4 weeks postexposure meal, with a 69% and 48% mean incidence of *R. felis* infection in trials 1 and 2, respectively (Table 1). The incidence of *R. felis* infection ranged from 30% to 100% and 10% to 90% within trials 1 and 2, respectively.

R. felis infection load was determined in *R. felis*–exposed flea groups by quantifying the copy number of *Rf*17kDa per individual flea lysate. The mean *R. felis* infection load per flea lysate was 1.52×10^6 and 1.24×10^6 rickettsiae per flea lysate in trials 1 and 2, respectively. *R. felis* infection load was significantly greater in fleas assessed at 28 dpe than at 7 dpe (Fig. 1). Specifically, trial 1 fleas had significantly greater *R. felis* infection loads at 21 and 28 dpe than at 7 dpe. In trial 2 similar trends of increasing *R. felis* infection load at the later assessment point with a significant increase only observed between 7 and 28 dpe. The range of the *R. felis* infection load (2.8×10^1 to 1.6×10^7) was expansive. Rickettsial gDNA was never detected in any control fleas fed blood meals with uninfected ISE6 cells.

The density of *R. felis* infection in individual fleas was determined by comparing the mean trial (±standard error of the mean) *Rf*17kDa/*Cf*18S ratio for each collection point. The mean *Rf*17kDa/*Cf*18S ratio (and range) was 0.54 (0.33–0.80) and 0.57 (0.35–0.71) for trials 1 and 2, respectively. In trial 1, the mean *Rf*17kDa/*Cf*18S ratio significantly increased from 7 to 14 dpe and remained significantly greater at all other collection points compared to 7 dpe (Fig. 2). In trial 2, a similar trend of increasing *R. felis* infection density was observed, but only fleas at 28 dpe had significantly greater rickettsial infection densities compared to fleas at 7 dpe (Fig. 2).

Detection of R. felis *in flea feces*

Total gDNA was extracted from samples of egg-free flea feces from each group and assessed at 2, 4, 7, 14, 21, and 28 dpe for fecal shedding of rickettsiae. Rickettsial gDNA was detectable in flea feces up to 28 dpe and was observed in most

Table 1. Incidence[a] of *Rickettsia felis* Infection in Cat Fleas Exposed to an *Rickettsia felis*–Infected Blood meal

	Trial 1	*Trial 2*
7 dpe	63% ± 13%	40% ± 15%
14 dpe	52% ± 19%	43% ± 24%
21 dpe	80% ± 12%	53% ± 13%
28 dpe	81% ± 12%	53% ± 15%
Mean	69%	48%

[a]Values are means ± standard error of the mean (10 samples per group) of *R. felis* infection incidence in fleas exposed to an *R. felis*–infected blood meal.

dpe, days postexposure.

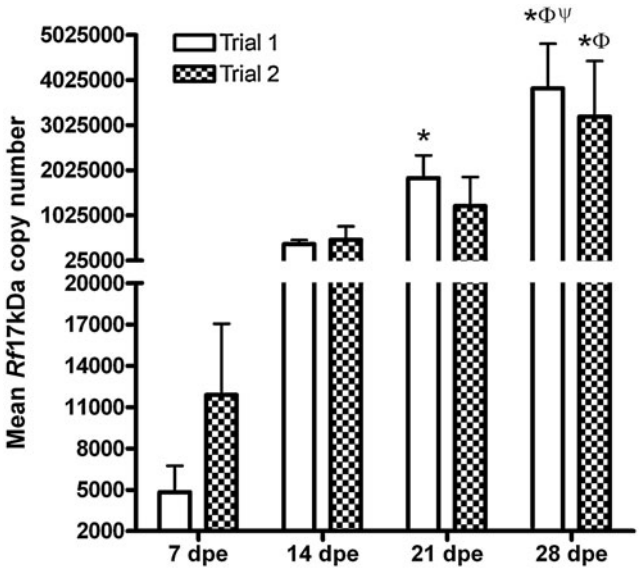

FIG. 1. Rickettsial load in fleas exposed to an *Rickettsia felis*–infected blood meal. In replicate trials, three groups of fleas were fed identical *R. felis*–infected blood meals for 1 day and 10 fleas per group were individually assessed at weekly intervals for *R. felis* infection load (*Rf*17kDa copy number per flea lysate) by quantitative real-time polymerase chain reaction. An overall mean of 1.52×10^6 and 1.24×10^6 rickettsiae per flea lysate was observed in trials 1 and 2, respectively. Bars represent weekly mean *Rf*17kDa copies per individual flea lysate, ±standard error of the mean. In both trials 1 and 2, a significant increase in the rickettsial load was observed in fleas assessed at the later time points. *Significant difference from 7 days postexposure (dpe), $^{\Phi}$significant difference from 14 dpe, and $^{\Psi}$significant difference from 21 dpe.

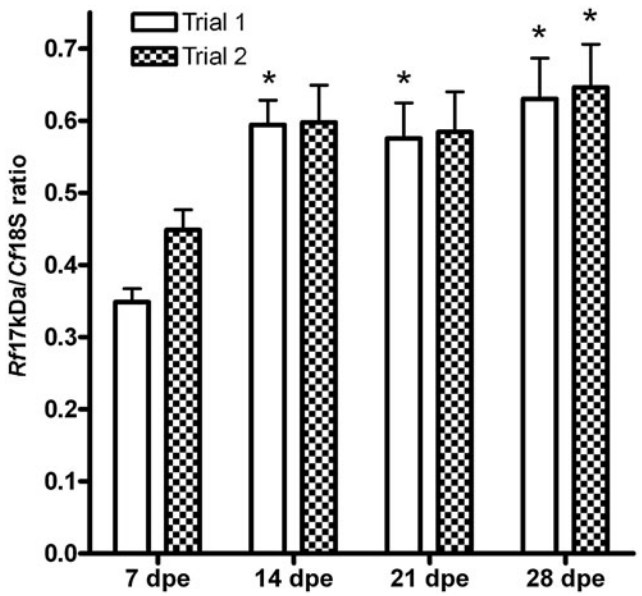

FIG. 2. Rickettsial infection density in fleas fed an *R. felis*–infected blood meal 7–28 dpe. The *R. felis* infection density was determined by quantitative real-time polymerase chain reaction of individual cat flea samples. Bars represent the mean (±standard error of the mean) *Rf*17kDa/*Cf*18S ratio of three experimental groups exposed to identical *R. felis* exposure meals for 1 day and assessed at weekly intervals in two replicate trials. Mean *R. felis* infection density was 0.54 and 0.57 in trials 1 and 2, respectively, and significantly greater density was observed in fleas sampled at later time points. *Significant difference from 7 dpe.

of the *R. felis*–exposed groups at each collection point. Significant variations in *R. felis* 17-kDa copy number in feces across collection time points were assessed by comparing the logarithmically transformed mean *R. felis* 17-kDa copy number per 50-mg flea feces at individual collection points. An initial significant decline in *R. felis* 17-kDa copies in flea feces was observed between 2 dpe (1.57×10^7 rickettsiae) and 4 dpe (1.7 rickettsiae). At 4 dpe rickettsial gDNA could only be detected in one group in trial 1 and no groups in trial 2. For both trials, mean rickettsial load detected in feces at 7, 14, 21, and 28 dpe was 4.37×10^5 rickettsiae per 50-mg feces. At these weekly collection points, rickettsial load did not vary significantly among groups within trials. The shedding pattern of individual *R. felis*–infected adult fleas was not assessed in this study. Feces from all control flea groups and environmental controls were consistently negative for *R. felis* DNA. At 9, 21, and 28 dpe in trial 1, the viability of *R. felis* in flea feces was assessed by amplification of *R. felis* 17-kDa cDNA synthesized from flea feces total RNA extracts. Rickettsial transcription of the 17-kDA antigen gene was detected in flea feces only at 21 dpe.

Vertical transmission of R. felis

F_1 progeny were collected as eggs or larvae, reared to adults, and assessed for *R. felis* infection. From batches of eggs (pooled by week postparental *R. felis*–infected blood meal) 10 unfed F_1 adults from every group were individually examined for vertically transmitted *R. felis* infection by qPCR amplification of a portion of the rickettsial 17-kDa antigen gene. Despite *R. felis* infection in adults, *R. felis* infection was not detected in any F_1 progeny.

Discussion

The present study demonstrates horizontal acquisition (with a resulting persistent infection) of *R. felis* by an arthropod feeding on an infectious blood meal. The present study demonstrates horizontal acquisition (with a resulting persistent infection) of *R. felis* by an arthropod feeding on an infectious blood meal. The absence of documented horizontal transmission of *R. felis* between arthropod and vertebrate hosts via blood feeding suggests an inability of arthropods to acquire *R. felis* infection from an infected vertebrate host and that vertical transmission between fleas is the only transmission route involved in the maintenance of *R. felis* in nature (Weinert et al. 2009). While the ability of *R. felis* to be transmitted between two hosts has yet to be unequivocally demonstrated, oral acquisition of a new infection by hematophagous arthropods is a perquisite to successful horizontal transmission.

Potential *R. felis* horizontal transmission mechanisms and routes have been examined previously, but were unsuccessful in infecting fleas. Cat fleas fed an *R. felis*–infected blood using an artificial host for 2–3 days and their progeny were PCR negative for *R. felis* infection (Wedincamp and Foil 2002). In

another experiment, uninfected fleas were fed on *R. felis* seropositive cats; however, all fleas and their progeny were negative for *R. felis* infection (Wedincamp and Foil 2002). The contrast of successful *R. felis* acquisition between earlier infection attempts and the current study may be due to the infectious meal preparation. Previous studies utilized undefined amounts of rickettsiae delivered in ground *R. felis*–infected flea lysates in nonheat-inactivated blood (Wedincamp and Foil 2002), compared to quantified cultures of *R. felis*–infected intact ISE6 cells fed to fleas in heat-inactivated blood in the current study. Heat inactivation of the delivery medium would limit the potential for complement-mediated lysis of rickettsiae in the preparation. In experiments using seropositive animals, lack of *R. felis* acquisition by fleas may be the result of minimal or already resolved infection in the animals (Wedincamp and Foil 2002), as a seropositive animal does not imply an active infection.

Interestingly, in an experiment on *R. felis* vertical transmission, *R. felis* prevalence waned (63%–2.5% over 12 generations) in fleas artificially fed uninfected blood meals, versus fleas fed on a cat host that retained a 65% *R. felis* infection prevalence (Wedincamp and Foil 2002), supporting the likelihood of flea acquisition of *R. felis* via a blood meal. Transmission of *R. felis* could require a minimum threshold infection in the vertebrate (e.g., during periods of bacteremia) for a feeding arthropod to ingest a sufficient amount of bacteria to become infected. The quantity of ingested bacteria necessary to result in arthropod infection can vary among bacterial species and strains. For example, *R. typhi* is acquired by feeding fleas during periods of rickettsemia (days 3–20 postinoculation) (Farhang-Azad et al. 1983), but requires ingestion of only a few rickettsiae for rat fleas to become infected and subsequently able to transmit *R. typhi* to another vertebrate (Vaughan and Azad 1990). Compared to *R. typhi*, acquisition and infection of fleas with *R. felis* likely requires a greater infection dose or host bacteremia. In the current study, fleas were exposed to a $\sim 8 \times 10^6$ *R. felis* per microliter of blood and were able to acquire a persistent *R. felis* infection. As fleas were allowed to feed freely on the *R. felis*–infected blood meal the specific infectious dose was not determined. Future studies are needed to define additional vertebrate-to-arthropod *R. felis* transmission parameters, including the minimum infectious dose.

In a previous study, *R. felis* infection load and density were examined over time in fleas naturally infected with *R. felis*, feeding on a cat host (Reif et al. 2008). Although the *R. felis* incidence was observed to decrease in the population over time during individual experiments, the *R. felis* infection load and infection density remained relatively stable with no association between increased rickettsial replication and flea reproduction observed. Despite a reduced mean infection density compared to vertically *R. felis*–infected fleas, fleas in the current study were able to acquire and develop a persistent *R. felis* infection for several weeks (similar to other pathogenic rickettsial species) after feeding on an infectious blood meal (Azad and Beard 1998).

The predominant route of transmission for most insect-transmitted pathogenic *Rickettsia* (e.g., *R. typhi* and *R. prowazekii*) is via fecal transmission (Azad 1990). As the cat flea is the only currently known biological vector of *R. felis*, the potential of horizontal transmission via fecal transmission must be considered. Previously, *R. felis* DNA was detected in the feces of cat fleas that fed on bovine blood containing *R. felis*–infected flea homogenates, but not human blood containing *R. felis* from culture 6 days post–*R. felis* meal (Wedincamp and Foil 2002). Acquisition of *R. felis* infection by flea larvae feeding on feces, flea eggs, and younger instar larvae positive for *R. felis* gDNA was also examined, but all resulting adults were negative for *R. felis* infection (Wedincamp and Foil 2002). In the current study, the potential for fecal transmission was also considered, as *R. felis* gDNA was detected in feces, not only directly after an *R. felis*–infected blood meal, but also up to 28 dpe. The high *Rf*17kDa copy number in the feces at 2 dpe reflects the large exposure dose of *R. felis* in the infected blood meals, followed by the significant decrease observed at 4 dpe as fleas finished digesting and excreting the exposure meal and continued to feed on uninfected blood. *R. felis*–positive feces at time points after 4 dpe were the result of rickettsial shedding by fleas, not contamination from a digested *R. felis*–infected blood meal as fleas were transferred into clean feeding cage several times during the study. The continued presence of *R. felis* gDNA in flea feces at later time points suggests active rickettsial infection. Differences in flea number and *R. felis* infection incidence in a group at any one time likely contributed to the variable presence of *R. felis* DNA observed in groups at each collection time point.

To address the viability of *R. felis* in flea feces, rickettsial RNA was amplified from flea feces. Due to limited sample, RNA was extracted from fecal samples only in trial 1 at 9, 21, and 28 dpe. Despite *R. felis* RNA being detected at only 21 dpe, the presence of viable *R. felis* in flea feces may indicate an additional transmission route. Further, the sensitivity of *R. felis* transcript detection in flea could be improved by targeting constitutively expressed transcripts (e.g., 16S rRNA). Fecal-borne transmission of other rickettsial species traditionally transmitted by saliva during arthropod feeding has also been studied. Under experimental conditions, *Rickettsia rickettsii* and *Rickettsia conorii* (both tick-transmitted rickettsial species) have been identified in louse feces by Immunofluorescence and PCR amplification of the rickettsial *ompA* gene (Houhamdi and Raoult 2006). As with *R. rickettsii* and *R. conorii* in lice feces, the viability and infectiousness of *R. felis* in flea feces is unknown and additional studies will be needed to determine if any of these rickettsial species, similar to *Bartonella* species, in insect feces are infectious to either arthropods or vertebrates.

Arthropods are the primary host of all rickettsial species and many rickettsial species are inherited and maintained via transovarial and transstadial transmission within arthropods (Sakurai et al. 2005). Vertical transmission of *R. felis* has been described in detail and is the primary transmission strategy in the LSU cat flea colony (Wedincamp and Foil 2002). Although *R. felis* acquisition and sustained infection in fleas was demonstrated in the current study, all F_1 progeny examined for vertically transmitted *R. felis* infection were negative. Potential factors for the lack of vertical transmission detection include the nature of the delivery preparation (*Rickettsia*-infected tick cells), the constitutive microbial profile of the cat fleas (Pornwiroon et al. 2007), or the rarity of vertical transmission events as an artifact of the *in vitro* model utilized.

The ability of cat fleas to acquire *R. felis* infection with subsequent development of a persistent infection after a single *R. felis*–infected blood meal is demonstrated. Acquisition of *R. felis* by this route results in mean infection densities less than that observed for vertically infected fleas. Further studies are

needed to examine the dissemination of horizontally acquired *R. felis* in cat fleas and other factors that could influence vertical transmission to progeny. Also, additional studies examining the viability and infectiousness of *R. felis* in flea feces are needed, as *R. felis* gDNA can be detected in flea feces almost 1 month after infection. These experiments confirm that cat fleas are able to acquire *R. felis* infection from an *R. felis*–infected blood meal and should serve as a platform to develop *in vivo* models of *R. felis* transmission between arthropod and vertebrate hosts.

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Disclosure Statement

No competing financial interest exists.

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