

Vertical transmission of *Rickettsia felis* in the cat flea (*Ctenocephalides felis* Bouché)

Jimmy Wedincamp, Jr. and Lane D. Foil

*Department of Entomology, Louisiana State University AgCenter
Baton Rouge, LA 70803-0001, U.S.A.*

Received 28 September 2001; Accepted 1 December 2001

ABSTRACT: *Rickettsia felis* can be maintained in cat fleas by vertical transmission for up to 12 generations without the benefit of an infected host. Horizontal transmission or the acquisition of *R. felis* by fleas feeding on cats or artificially infected meals were not demonstrated in this study. Horizontal transmission of *R. felis* by the ingestion of feces or eggs by flea larvae was not detected. We also tested for and found no evidence to support horizontal transmission by contact among positive fleas and negative fleas. ***Journal of Vector Ecology* 27 (1): 96-101. 2002.**

Keyword Index: *Ctenocephalides felis*, *Rickettsia felis*, cats, infection.

INTRODUCTION

Rickettsia felis is an endosymbiont of the cat flea (*Ctenocephalides felis* Bouché) that is distinguishable from *R. typhi* by polymerase chain reaction (PCR) (Adams et al. 1990). Higgins et al. (1994) reported that fleas from eight separate cat flea colonies located throughout the United States were found to be infected with *R. felis*; infection rates up to 93% were described. Three of the colonies were initiated from a common source (E1 Labs in Soquel, California) but five of the colonies were initiated from wild-caught fleas.

There have been no studies on the mechanisms by which *R. felis* infection rates of up to 93% in flea colonies are maintained. All of the flea colonies that were tested by Higgins et al. (1994) were maintained on cat hosts, but no studies have been conducted to determine if cats are an infectious source of *R. felis*. Adams et al. (1990) reported that *R. felis* was found in ovarian tissue of fleas, which would indicate potential vertical transmission in fleas; however, the mechanisms of transmission of *R. felis* among cat fleas has not been determined. This study was conducted to determine how *R. felis* is maintained within cat flea lines and to determine if routes of transmission to other flea lines exist.

MATERIALS AND METHODS

PCR procedures for *R. felis* DNA flea samples followed previously published techniques (Azad et al. 1992, Wedincamp and Foil 2000). Fleas were assayed by PCR (Azad et al. 1992) for the presence of *R. felis*

DNA using the 17 kDa primer. Restriction fragment length polymorphism (RFLP) analysis of the PCR products that were amplified indicated they were of *R. felis* origin. Likewise, DNA sequence data comparisons indicated that the PCR products that were amplified had a 99% homology to *R. felis* (Genbank accession number M82878). The indirect immunofluorescent-antibody (IFA) assay was used to detect antibody to *R. felis-typhi* in cat serum samples (Wedincamp and Foil 2000).

Laboratory colonies of cats and cat fleas (*Ctenocephalides felis* Bouché) were maintained at the Louisiana State University AgCenter Research Station (Henderson and Foil 1993). Guidelines issued by the Louisiana State University Institutional Animal Care and Use Committee were followed in maintaining the experimental cats. The flea colony maintains an average infection rate of approximately 65% with *R. felis* (Wedincamp and Foil, 2000). Fleas (*Ctenocephalides felis* Bouché) obtained from the Heska Corporation (Fort Collins, CO) flea colony were determined to be free of *R. felis* by PCR assay. For colony maintenance, flea larvae were maintained at 80% humidity in containers with sand and approximately 1 g of spray dried beef blood and 0.5 g of brewers yeast.

Vertical transmission

Fleas originating from the Louisiana State University (LSU) AgCenter colony were maintained through 12 generations (hereafter referred to as lab colony) using a Rutledge blood feeding system (Rutledge et al. 1964). The adult fleas were fed bovine blood that was PCR negative for *R. felis*. Flea eggs were collected each

generation and placed in rearing containers. After 10 d the pupae were strained from the sand and placed in 450 ml jars until adult fleas emerged. Forty adult fleas from each generation were individually assayed for *R. felis* DNA by PCR.

Daily vertical transmission

Three groups of LSU AgCenter fleas (ca 75 adults per group) were placed into each of three Rutledge chambers and fed bovine blood. Eggs were collected daily from each feeder for 5 d and placed in individual containers. After emergence, infection rates of the adult fleas were determined by PCR assay.

Approximately 50 LSU AgCenter fleas were placed on each of 3 cats maintained at that facility and allowed to feed for 5 d. All other fleas had been removed from the cats by thorough combing prior to this study. Eggs were collected daily from pans located beneath the cats (Henderson and Foil 1993) and then placed in individual containers. After adult emergence, the fleas were individually tested for *R. felis* DNA by PCR. A Chi-square test was used to compare proportions of *R. felis* positive and negative fleas between treatments.

Horizontal transmission

Heska larvae were placed individually into 15 x 100 mm test tubes with LSU AgCenter flea eggs plus dried blood and yeast (60 larvae) or flea feces (PCR positive) plus yeast (110 larvae). A group of 100 control larvae also was maintained on spray-dried beef blood and brewers yeast. The flea larvae were held until adult eclosion and then assayed by PCR for the presence of *R. felis* DNA.

LSU AgCenter adult fleas were paired with Heska adult fleas: 5 LSU AgCenter males with either 15 Heska females or 15 LSU AgCenter females and 5 Heska males with either 15 Heska females or 15 LSU AgCenter females. There were three replicates of each group. The fleas were maintained on the Rutledge system for 5 d, and then tested by PCR analysis for horizontal transmission of *R. felis*. The eggs produced by Heska females were reared to the adult stage and assayed by PCR.

Artificially infected blood meals

There were three trials in this experiment. Approximately 200 newly emerged Heska fleas were provided human blood containing 10^9 PFU/ml *R. felis-typhi* from tissue culture (Radulovic et al. 1995). The fleas were fed for 6 h on two consecutive days using a commercially available artificial host (artificial dog; Wade and Georgi, 1988) from FleaData® (Farmington, NY). The fleas were then fed uninfected blood for the

next 6 d. Another group of ca 200 Heska fleas was fed defibrinated cat blood containing 10^9 PFU/ml *R. felis-typhi* from tissue culture using an "artificial dog" for 2 h on three consecutive days. The fleas were then fed cat blood for the next 6 d. A third group of Heska fleas was provided bovine blood containing *R. felis* from ground flea homogenate for 5 d using the Rutledge system. The flea homogenate was prepared by grinding 50 adult LSU AgCenter fleas in 1.5 ml of saline buffer (Takken 1980). In each trial, eggs were collected daily and reared until adult emergence. PCR analysis was conducted on both the fed adults and their progeny.

Fleas fed on cats

There were three trials in this study. In the first trial approximately 75 Heska fleas were fed on each of three cats (SG1- SG3) for 5 d using the chambered flea technique (Thomas et al. 1996). The cats had been continually exposed to *R. felis* infected fleas for at least 12 months prior to this study and were determined to be seropositive by IFA.

In a second trial, approximately 75 Lab Colony fleas from generation f8 were fed on two cats (SG2 and SG3), and one month later 75 Lab Colony fleas from generation f9 were fed on SG2 and SG4 for 5 d using the chambered flea technique. The infection rate of the Lab Colony fleas maintained on the artificial membrane system was 2.5 and 5.0 % for f8 and f9, respectively.

In a third trial, four specific pathogen free cats (SPF1-SPF4; *R. felis* negative and flea naïve) were introduced into the LSU AgCenter cat colony and infested with approximately 75 LSU AgCenter fleas twice per week throughout the trial. The SPF cats were housed individually in the same building with the LSU AgCenter cat colony. Approximately two weeks after the cats were infested, 75 adult Lab Colony (f10; infection rate 22.5%) fleas were fed on the cats for 5 d using the chambered flea technique. The produced eggs (equivalent to f11) were reared to adults and fed on the same cats as their parents. At this time, SP1 was removed from the study; adult fleas that fed on SP1 and their progeny were assayed for *R. felis* by PCR. The eggs from SP2-SP4 (equivalent to f12) were reared to adults and fed on the same cats as their parents. Adult fleas that fed on the three cats and their progeny were assayed for *R. felis* by PCR. The cats were tested by IFA monthly.

RESULTS

Vertical transmission

The Lab Colony fleas strain maintained infection with *R. felis* through 12 generations (Table 1). There was a decline in incidence through the F8 generation to 2.5%,

Table 1. *R. felis* DNA detected in cat fleas fed bovine blood via an artificial membrane system through 12 generations.

Generation	Infection Rate
F1	25/40 (63%)
F2	17/40 (43%)
F3	15/40 (38%)
F4	6/40 (15%)
F5	4/40 (10%)
F6	2/40 (5%)
F7	1/40 (2.5%)
F8	1/40 (2.5%)
F9	2/40 (5%)
F10	9/40 (22.5%)
F11	5/40 (12.5%)
F12	1/40 (2.5%)

followed by an increase at the F 10 generation to 22.5%. This increase was followed by a decrease to 2.5% by the F12 generation. PCR assays conducted on Heska colony fleas, using the same reagents and laboratory facilities, were consistently negative, indicating that contamination of assays was not responsible for positive results. The incidence of *R. felis* in the LSU AgCenter flea colony remained at approximately 65% throughout the study.

Daily vertical transmission

There was no statistical difference (at the 0.05 level of significance) between the daily rate of transovarial transmission of *R. felis* in the LSU AgCenter fleas fed

on cats and bovine blood *in vitro* (Table 2). Similarly, the average infection rate of the progeny of fleas fed on cats or bovine blood was not significantly different.

Horizontal transmission

A sample of adult fleas from the LSU AgCenter at the time of these studies had an average infection rate of 65% (42/65) for *R. felis* when assayed by 17 kDa PCR. When Heska larvae were allowed to feed on the eggs and feces produced by LSU AgCenter fleas (samples of the eggs and feces were positive for *R. felis* DNA by PCR assay), all 170 adults that developed were negative for *R. felis* by PCR. The 100 control fleas also were negative for *R. felis* DNA.

When Heska and LSU AgCenter fleas were placed together, no transmission of *R. felis* from LSU AgCenter fleas to Heska fleas by copulation or contact was detected by 17 kDa PCR. The progeny of the Heska females mated to LSU AgCenter fleas also were negative for *R. felis* DNA.

Artificially infected blood meals

All of the adult fleas fed artificially infected blood for 2-3 days and then fed negative blood for 6 days, as well as their progeny, were PCR negative. *R. felis* DNA was detected in the feces produced by fleas fed bovine blood containing flea homogenate. *R. felis* DNA was not detected in the feces from the fleas fed on human blood; feces were not collected from fleas fed on cat blood.

Fleas fed on infected cats

Heska fleas fed on three cats (SG1-SG3) were negative for *R. felis* as were their progeny. Five percent

Table 2. *R. felis* DNA detected in the progeny of fleas fed on bovine blood *in vitro* or on cats. Sample size was ten unless limited by available specimens.

	Daily Infection Rate					Avg. Infection Rate
	24hr	48hr	72hr	96hr	120hr	
Feeder 1	4/10	4/10	5/10	9/10	9/10	31/50 (62%)
Feeder 2	1/7	5/10	6/10	6/9	4/10	22/46 (48%)
Feeder 3	6/10	7/10	6/10	7/10	6/10	32/50 (64%)
Cat 1	6/10	7/10	2/4	8/10	7/10	30/44 (68%)
Cat 2	4/10	9/10	9/10	6/10	4/10	32/50 (64%)
Cat 3	5/10	7/10	8/10	9/10	6/10	35/50 (70%)

Table 3. Attempted horizontal transmission of *R. felis* to fleas fed on cats through three generations. The fleas were assayed at the second generation on cat SPF1 due to the removal of the cat from the study in month two. The infection rate of the equivalent f11 lab strain fleas fed on the artificial membrane system was 12.5%.

Cat	Month Seropositive	Flea Source	PCR Assay of 3rd Generation Progeny
SPF1	2nd	lab	1/10
SPF2	3rd	lab	0/10
SPF3	2nd	lab	0/10
SPF4	2nd	lab	0/10

of Lab Colony fleas (f8) fed on cats were PCR positive for *R. felis*, but none of their progeny were positive. All Lab Colony fleas (f9) were negative for *R. felis* after feeding on cats and all of their progeny were PCR negative.

All SPF cats were determined to be seronegative prior to the first flea feeding (Lab Colony, f10), but all of four cats were seropositive by the end of the study (Table 3). No horizontal transmission of *R. felis* to fleas fed on cats for three generations was detected. Samples of the progeny from the third generation that fed on SPF2-SPF4 were all negative for *R. felis*. The equivalent f12 lab strain fleas fed on the artificial membrane system had an infection rate of 2.5%. The progeny from the second generation of fleas fed on cat SPF1 were assayed and one flea was positive. The infection rate of the equivalent f11 lab strain fleas fed on the artificial membrane system was 12.5%.

DISCUSSION

We tested for vertical and horizontal transmission of *R. felis*. For vertical transmission, we demonstrated that *R. felis* can be maintained for up to 12 generations without the benefit of an infected blood meal. The flea infection rate did fall when compared to that of LSU AgCenter fleas on cats that maintained a relatively constant 65% infection rate. One explanation for the infection rate of the LSU AgCenter fleas on cats remaining stable while decreasing through time when fed *in vitro* on bovine blood would be that the cats were a source of infection for the fleas. However, the acquisition of *R. felis* by fleas feeding on cats was not demonstrated in this study. When uninfected fleas fed on cats that had been in the colony for over one year, there was no transmission of *R. felis* to the fleas.

An alternative explanation for the relatively constant infection rate in fleas at the LSU AgCenter cat colony would be the occurrence of occasional rickettsemias in the cats. In this manner, fleas would get an infective blood source intermittently to sustain infection rates in the colony. However, all specific pathogen free (SPF) cats in our feeding study were seropositive for *R. felis-typhi* by the second month of the study and may have been rickettsemic at some time during the study, but no infections were detected in the progeny of fleas fed on these cats. Additionally, neither fleas fed artificially infected blood meals nor their progeny acquired infections. Although the feces of the fleas that were fed the infected flea homogenate did test positive by PCR assay, this may indicate that the *R. felis* DNA found in the flea homogenate was simply passed through the flea gut and did not establish an infection in the flea.

Flea larvae have been shown to feed on the feces and eggs deposited by adult fleas (Lawrence 1995). With this abundant food supply available to developing flea larvae, the possibility of flea larvae acquiring *R. felis* by the ingestion of an infected meal was a likely route to study. However, horizontal transmission of *R. felis* by the ingestion of feces or eggs by flea larvae was not detected. We also tested for and found no evidence to support horizontal transmission by contact among positive fleas and negative fleas.

The maintenance of *R. felis* in cat fleas can be compared to the maintenance of *Orientia tsutsugamushi* in Leptotrombidium mite reservoirs. Even though the mite is capable of maintaining the agent transovarially (Takahashi et al. 1988) there has been no success reported in attempts to infect naive mites (*Leptotrombidium deliense*, *L. fletcheri*, and *L. arenicola*) by feeding *O. tsutsugamushi*-infective meals (Walker et al. 1975). It is currently believed that the mites serve as both the vector

and reservoir of *O. tsutsugamushi*. Similarly, *R. akari* is transmitted by the house mouse mite (*Liponyssoides sanguineus*) and can be maintained in mite populations transovarially (Burgdorfer and Varma 1967). In this respect, cat fleas may serve as both the vector and host of *R. felis*. The fact that this organism can be maintained for 12 generations without the benefit of an infected host indicates that the maintenance of *R. felis* in cat fleas is principally by transovarial transmission. In contrast to the reported detrimental effects to ticks (engorged female death and lowered hatch rates) that pass *R. rickettsii* for more than 5 generations (Burgdorfer 1975), we detected no detrimental effects, i.e. lowered egg production, on cat fleas maintaining *R. felis* for numerous generations (unpublished data).

There are alternate routes for the acquisition of infectious agents by insects. One example is the enhanced infection in mosquitoes that concurrently ingest microfilariae and arboviruses. Turell et al. (1984) found increased transmission of Rift Valley Fever virus to gerbils by mosquitoes that had ingested blood meals that were concurrently infected with *Brugia malayi* microfilariae and Rift Valley Fever virus. There are filarids and cestodes also associated with fleas. Fleas can serve as an invertebrate host for *Dipylidium caninum* and vector of *Dipetalonema reconditum* (Harwood and James 1979). Thus, enhanced infection for *R. felis* of fleas by concurrent parasite infection remains a possibility in the maintenance of *R. felis* in fleas.

Vertical transmission is a route by which *R. felis* is maintained in successive generations of cat fleas. This study was the first to show that *R. felis* is maintained in cat flea lines without need for a vertebrate reservoir. If ingestion of *R. felis* is not a route for infection of naive flea lines, then possible fitness differences or mating incompatibilities between positive and negative fleas might account for the high incidence of *R. felis* in certain flea colonies.

Acknowledgments

The authors thank James Higgins, USDA-ARS, and Abdu Azad, University of Maryland Medical School, for providing technical assistance and manuscript review. Approved for publication by the Director of the LAES as publication number 01-17-0578.

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