Rickettsia felis Infection in the Cat Flea (Siphonaptera: Pulicidae)[1]

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Abstract We compared fecundity and eclosion rates of two cat flea *Ctenocephalides felis* (Bouché) lines, one infected with *Rickettsia felis* and the other not infected. Egg and feces production were compared between the two flea lines fed on cats; statistical differences were found in feces production ($P = .0057$) but not egg production ($P = 0.1081$). Cross-mating studies also were performed; no reduction in fecundity was found when males infected with *R. felis* were mated with uninfected females. Additionally, the infected flea line had a statistically higher eclosion rate ($P = 0.0030$) than the non-infected flea line.

Key Words *Rickettsia felis, Ctenocephalides felis,* cats, infection

Rickettsia felis is an endosymbiont of the cat flea, *Ctenocephalides felis* (Bouché). *Rickettsia felis* has been recovered from wild-caught fleas and in laboratory colonies throughout the United States; however, several of these laboratory colonies were established with fleas from a single source (Higgins et al. 1994). The mechanisms by which *R. felis* is introduced into naive flea populations and reaches high infection rates (up to 93%) are unknown.

Wedincamp and Foil (2000) reported 65% of cat fleas were infected with *R. felis* in the laboratory cat flea colony at the Louisiana State University Agricultural Center, St. Gabriel Research Station (LSU AgCenter). The authors also demonstrated transovarial transmission of *R. felis* for 12 generations with fleas that were fed blood meals that did not contain *R. felis*. They were unable to demonstrate horizontal transmission (Wedincamp and Foil 2002).

Because horizontal transmission of *R. felis* has not been demonstrated, and *R. felis* is maintained in cat flea populations at high infection levels, fitness differences and/or mating incompatibilities may exist. Higgins et al. (1994) speculated that *R. felis* might affect cat flea biology by such mechanisms. The purpose of this investigation was to determine possible effects of *R. felis* on cat flea biology.

Materials and Methods

Comparisons were made using two laboratory flea colonies. One colony (LSU AgCenter) was maintained at the LSU AgCenter (Henderson and Foil 1993). Approxi-

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mately 65% of the fleas in this colony were infected with *R. felis* (Wedincamp and Foil 2000). The other colony was maintained at Heska Corporation (Fort Collins, CO) and did not contain *R. felis* as determined by the polymerase chain reaction (PCR) assay.

Fleas were assayed via PCR (Azad et al. 1992) for the presence of *R. felis* DNA using the 17 kDa primer. The 17 kDa primers are specific only to the generic level, thus confirmation that products were of *R. felis* origin was made by restriction fragment length polymorphism (Azad et al. 1992) and DNA sequencing. The 17 kDa PCR product amplified from the fleas was cloned and sequenced using the Invitrogen T/A cloning kit (San Diego, CA), as described by Higgins et al. (1996).

***In vitro* study.** Fecundity comparisons of the LSU AgCenter and Heska flea colonies were made *in vitro.* The flea colonies were maintained by feeding adults for 7 d on bovine blood once each month during April, May, and June. Adult fleas were anesthetized by chilling at −20°C for 3 min, sorted by sex, then placed into feeding cages. Each cage contained 50 females and 25 males, and there were 3 cages per colony. Adult fleas were fed bovine blood containing 14 mM sodium citrate using the Rutledge system (Rutledge et al. 1964). Eggs and feces were collected in a 450-mm diam glass Petri dish attached to the bottom of each flea feeder. The feces from each individual feeder were collected, weighed, and the amount of feces per day per female was calculated. Eggs were aspirated from feeders, counted, and the number of eggs produced per female per day was calculated.

***In vivo* study.** Fecundity comparisons of the LSU AgCenter and Heska colony fleas were made *in vivo* by feeding on three cats. The fleas were fed on the cats for 5 d once each month during April, May, and June. Adult fleas were anesthetized by chilling at −20°C for 3 min, sorted by sex, then placed into separate feeding cages (Thomas et al. 1996), and a cage of each colony was placed on each of the three cats. Each cage contained 50 females and 25 males. The feces from each individual feeder were collected and weighed. The weight of feces per female per day was calculated. Eggs were also aspirated from feeders, counted, and the number of eggs produced per female per day calculated.

Cross-mating study. Cross-mating tests of Heska and LSU AgCenter fleas were also conducted. The cross-matings involved four treatments (pair matings) with three replicates each. LSU AgCenter fleas (65% infection rate) were paired with uninfected Heska fleas in the following manner: 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. Fleas were maintained in the respective treatments and fed bovine blood on the Rutledge system for 5 d, and daily egg and feces production was recorded.

Adult eclosion study. In a separate trial, adult eclosion rates were compared. One hundred eggs from each colony (LSU AgCenter and Heska) were placed into individual 30-mL plastic snap cap tubes containing 15 g of sand, 1 g of spray-dried beef blood, and 0.5 g of Brewers yeast. Three replications were performed. The containers were held for approximately one month and then adult fleas were counted.

Total egg and feces production and eclosion rates were compared using analysis of variance (ANOVA) and the least significant difference means separation test [(LSD) (SAS Institute 1987)].

Results and Discussion

The number of eggs and amount of feces produced by the Heska and LSU AgCenter colony fleas fed bovine blood *in vitro* is summarized in Table 1. Egg production

Table 1. Mean (±SD) eggs and feces produced by Heska and LSU AgCenter colony fleas fed bovine blood *in vitro*

Colony	# eggs/flea/d Mean ± SD	mg feces/flea/d Mean ± SD
Heska	3.3 ± 0.8a	2.0 ± 0.8a
LSU AgCenter	0.3 ± 0.2b	0.4 ± 0.2b

Means within columns followed by the same letter were not significantly different ($P \geq 0.05$) by LSD.

for the LSU AgCenter colony was significantly lower than for the Heska colony fleas ($P \leq 0.001$). Also, LSU AgCenter colony feces production was lower than that of Heska ($P \leq 0.001$). LSU AgCenter colony fleas began producing eggs on the third day of feeding while Heska colony fleas began producing eggs on the second day of feeding.

Egg production of Heska and the LSU AgCenter colony fleas fed *in vivo* on cats was not significantly different ($P = 0.1081$) (Table 2). However, the amount of feces produced by Heska fleas was higher than that of the LSU AgCenter colony ($P = 0.0057$). Egg production for both LSU AgCenter and Heska flea colonies began on the second day of feeding.

In cross-mating studies using the *in vitro* system, we found egg production of Heska females was significantly higher than egg production of LSU AgCenter females regardless of the males with which they were mated ($P = 0.0013$) (Table 3). There was no statistical difference between the egg production of Heska colony females when mated to LSU AgCenter males or Heska colony males ($P = 0.2524$). However, matings between Heska colony females and LSU AgCenter colony males averaged 19% fewer eggs than Heska colony female and Heska colony male matings. Similarly, LSU AgCenter colony females produced 23% fewer eggs when mated to Heska colony males rather than LSU AgCenter colony males.

The adult eclosion study showed that the LSU AgCenter colony had an average adult emergence rate of 62% and the Heska emergence rate was 38%. The Heska colony fleas had a statistically lower adult emergence rate than the LSU AgCenter colony ($P = 0.0030$) (Table 4).

We previously demonstrated that *R. felis* is not transmitted by ingestion of potentially contaminated blood meals or by contact with infected individuals (Wedincamp and Foil 2002) and that *R. felis* is vertically transmitted. If fleas do not acquire *R. felis*

Table 2. Mean (±SD) eggs and feces production by LSU AgCenter and Heska colony fleas fed on cats

Colony	# eggs/flea/d Mean ± SD	mg feces/flea/d Mean ± SD
Heska	13.8 ± 6.6a	5.2 ± 2a
LSU AgCenter	9.6 ± 3.5a	2.5 ± 0.7b

Means within columns followed by the same letter were not significantly different ($P \geq 0.05$) by LSD.

Table 3. Mean (±SD) egg production of LSU AgCenter and Heska cross-matings

Treatment	Mean ± SD	# eggs/female/d
LSU females X LSU males	93 ± 60.65	1.33a
Heska females X Heska males	328 ± 122.72	4.80b
Heska females X LSU males	254 ± 12.29	3.72b
LSU females X Heska males	76 ± 53.87	1.06a

Means followed by the same letter were not significantly different ($P \geq 0.05$) by LSD.

Table 4. Mean (±SD) number of adult cat fleas that eclosed per 100 eggs

Flea colony	# adult fleas that eclosed per 100 eggs Mean ± SD
LSU AgCenter	62.3 ± 10.4a
Heska	38.3 ± 14.5b

Means with the same letter were not significantly different ($P \geq 0.05$) by LSD.

by ingestion or contact, then other mechanisms may occur to explain infection levels near 100% in some flea colonies (Higgins et al. 1994).

One possible explanation may be that infected fleas have a selective advantage over non-infected individuals, i.e., increased fecundity, higher adult eclosion rates. When comparing the egg and feces production for colonies of fleas fed *in vitro* on bovine blood, we found that the LSU AgCenter colony did not produce as many eggs or as much feces as the Heska colony. In addition, production of eggs by the LSU AgCenter flea colony began on the third day of feeding, whereas egg production of the Heska colony fleas began on the second day of feeding.

When the same comparisons were made *in vivo* on cat hosts, there were no significant differences in egg production between LSU AgCenter and Heska colony fleas. There was, however, a statistical difference between feces production of Heska and LSU AgCenter colony fleas with the LSU AgCenter colony producing 32% less feces per egg than the Heska flea colony. This may indicate that the LSU AgCenter flea colony is more fecund than the Heska flea colony. This difference could translate into less feeding by the LSU AgCenter flea colony with equal egg production. Less feeding by the flea means less irritation to the host. Because cats have been shown to remove 49.5% of the fleas released onto them by grooming (Wade and Georgi 1988), it would seem logical that fleas capable of producing eggs while feeding less would be detected less often than fleas requiring more blood. Egg production for both colonies began on the second day when fed on cats. These data indicate that *R. felis* infected fleas (LSU AgCenter colony) do equally as well as uninfected fleas (Heska colony) when fed on cats, while producing less feces.

The differences we observed *in vitro* may be due to a selection process to the *in vitro* feeding system and not related to the infection status of the fleas. One major

difference between *in vitro* and *in vivo* blood meals is the presence of anticoagulants in the *in vitro* blood meal. The LSU AgCenter colony fleas have been shown to produce fewer eggs as the concentration of sodium citrate is increased (Lawrence 1995). Similarly, Guerrero et al. (1993) found that horn fly, *Haematobia irritans* (L.), egg production decreased as sodium citrate concentrations increased. There is the possibility that differences we observed are due to genetic differences caused by selection to the artificial membrane system and not related to the presence of *R. felis.* The colony maintained at Heska Corp. has been maintained on the artificial membrane system for over 100 generations (Rex Thomas, pers. commun.). The LSU AgCenter flea line has been maintained on cats for over 8 yrs. Artificial rearing techniques have been found to affect the biology of insects by placing selection pressures on them that would normally not be encountered in nature. For example, Bush et al. (1976) found that the rearing techniques for screwworm, *Cochliomyia hominivorax* (Coquerel), could possibly affect the competitive ability of colony males to mate with females in nature. Determining whether the *in vitro* selection process was coincidental to the reduced incidence of *R. felis* could be the subject of future studies.

In the cross-mating studies we found no statistical difference between egg production of the Heska colony females when mated to LSU AgCenter males versus Heska males; however, there was a 19% reduction in the number of eggs produced. Findings of reduced fecundity in females mated to males infected with *Wolbachiae* have been reported for other insects (O'Neill et al. 1993). This may not be the case in our study because the reciprocal cross (LSU AgCenter females and Heska males) also indicated a reduction in fecundity.

We also found that the Heska colony fleas had a statistically lower emergence rate than the LSU AgCenter colony. This phenomenon could have an effect on the ratios of infected and non-infected individuals in a mixed population.

In conclusion, we found that LSU AgCenter fleas produce as many eggs as the Heska fleas when fed on cats but produce 32% less feces, and that LSU AgCenter fleas have a statistically higher eclosion rate. These observations indicate that *R. felis* may affect infection rates of localized flea populations by causing fleas infected with *R. felis* to increase as a proportion of the total flea population. Further studies need to be conducted to verify the lack of mating incompatibilities caused by infection with *R. felis.*

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