Culling of Urban Norway Rats and Carriage of Bartonella spp. Bacteria, Vancouver, British Columbia, Canada

Kaylee A. Byers, Michael J. Lee, Janet E. Hill, Champika Fernando, Laura Speerin, Christina M. Donovan, David M. Patrick, Chelsea G. Himsworth

We investigated the effects of culling on Bartonella spp. bacteria carriage among urban rats in Canada. We found that the odds of Bartonella spp. carriage increased across city blocks except those in which culling occurred. Removing rats may have prevented an increase in Bartonella spp. prevalence, potentially lowering human health risks.

Urban Norway rats (Rattus norvegicus) carry Bartonella spp., which are bacteria transmitted among rats and to humans through vectors including fleas (1). Infection in humans can result in fever, fatigue, myalgia, and endocarditis (2). In Vancouver, British Columbia, Canada, a serosurvey of residents of an underresourced neighborhood found that 3% of participants had been exposed to B. tribocorum (3), a species found in rats in this neighborhood (4), suggesting that rats may be an exposure source for humans in this area.

Although aimed at decreasing disease risks, culling methods (i.e., lethal removal) may increase zoonotic pathogen prevalence by altering normal behaviors that modify pathogen transmission (5,6). We sought to determine whether culling rats altered Bartonella spp. prevalence in rats and their fleas in the Downtown Eastside neighborhood of Vancouver. The University of British Columbia’s Animal Care Committee (A14-0265) approved study procedures.

The Study

We trapped rats in 12 study sites (5 intervention, 7 control), each comprising 3 contiguous city blocks (36 total blocks) (Figure, panel A) during June 2016–January 2017 (Appendix, https://wwwnc.cdc.gov/EID/article/28/8/21-1164-App1.pdf). We placed 10 live traps (Tomahawk Live Traps, https://www.livetrap.com) in the alley of each block. We conducted the experiment in 3 trapping phases: before, during, and after the intervention (Figure, panel B). Before and after the intervention, we captured rats, gave each a numbered ear tag, and released it to its capture site. In the center block of intervention sites culling occurred during the second trapping phase. In flanking blocks (those adjacent to the intervention block) and control blocks, no culling occurred (Figure, panel A).

We collected blood from all rats via jugular puncture under isoflurane anesthesia. We collected fleas by brushing the coat.

We identified fleas to species (7), and pooled ≤5 fleas per rat. We extracted DNA from rat blood and fleas using the DNEasy Blood and Tissue Kit (QIAGEN, https://www.qiagen.com). We tested DNA extracts for Bartonella spp. by real-time PCR. For rat blood, we used primers to detect a 380-bp segment of the citrate synthase gene (gltA) (8). For fleas, we used a probe-based real-time PCR assay to detect a 302-bp fragment of the ssrA gene (9). We conducted our analysis as described in Himsworth et al. (10).

We used generalized linear mixed models to assess the relationship between the intervention and Bartonella spp. carriage. We found that the odds of Bartonella spp. carriage increased across city blocks except those in which culling occurred. Removing rats may have prevented an increase in Bartonella spp. prevalence, potentially lowering human health risks.

Author affiliations: The University of British Columbia School of Population and Public Health, Vancouver, British Columbia, Canada (K.A. Byers, M.J. Lee, C.M. Donovan, D.M. Patrick); The University of British Columbia, Vancouver (K.A. Byers, C.G. Himsworth); Canadian Wildlife Health Cooperative, Abbotsford, British Columbia, Canada (K.A. Byers, M.J. Lee, C.G. Himsworth); University of Saskatchewan, Saskatoon, Saskatchewan, Canada (J.E. Hill, C. Fernando, L. Speerin); British Columbia Centre for Disease Control, Vancouver (D.M. Patrick); British Columbia Ministry of Agriculture, Abbotsford (C.G. Himsworth)

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1These first authors contributed equally to this article.
ella spp. carriage. We controlled for spatial clustering by city block as a random effect. We assessed positive or negative carriage by rats (model A) and fleas (model B) and the number of fleas per rat (model C). We analyzed carriage models A and B by logistic regression and model C by negative binomial regression. For all models, the intervention variable consisted of 4 categories indicating when rats or fleas were caught: before the intervention in all blocks; after the intervention in control blocks; after the intervention in flanking blocks; and after the intervention in intervention blocks.

We used a hypothesis-testing model building approach to estimate the effect of the intervention while accounting for covariates (Table). We retained covariates if they confounded the relationship between the intervention and the outcome (i.e., if they changed the effect of any level of the intervention by $\geq10\%$ or if their association with the outcome and intervention had $p\leq0.25$). We also kept independent predictors of the outcome if they significantly improved the model, as indicated by a likelihood ratio test result of $p\leq0.05$; that test compared 2 nested models, each with the intervention variable and all confounders present, but with and without the potential predictor variable.

We trapped 512 Norway rats; 206 (40.2%) of them had fleas. The median number of fleas per rat was 0 (range 0–58; mean 1.18). All fleas were Nosopsyllus fasciatus. We obtained blood from 454 rats; 90 (20%) tested positive for Bartonella spp. We tested 201 flea pools; 86 (42.8%) tested positive for Bartonella spp. (Table).

In the final model A, which contained the variables season, presence of Bartonella spp.–positive fleas, and wound presence as covariates, the odds of Bartonella spp. carriage were significantly higher among rats caught after the intervention in control blocks (odds ratio [OR] 2.68; 95% CI 1.22–6.67) and flanking blocks (OR 7.26; 95% CI 1.56–38.17), but not in the intervention blocks (OR 2.03; 95% CI 0.22–15.41), when compared with the odds of carriage before the intervention in all block types (Table). We saw no association between the intervention and the number of fleas per rat or Bartonella spp. carriage by fleas.

Conclusions

The prevalence of Bartonella spp. bacteria among rats in this neighborhood has been shown to increase in...
the fall (4). Our study suggests that culling rats may have prevented this increase within the blocks where culling occurred.

Removing rats may change how individual rats interact within colonies, which alters pathogen transmission. Bartonella spp. transmission via fleas (1) requires close contact among individual rats. Rats burrow communally, establishing a network of chambers with some shared nests (11). Those nests promote close contact among rats and act as a source of fleas that spend time in the nest (12). Decreased rat population density may lessen nest sharing and behaviors such as social grooming, thereby reducing opportunities for fleas to transmit Bartonella spp. among individual rats. A reduction in Bartonella spp. prevalence may decrease exposure risk for humans, but the relationship between rodents, vectors, pathogens, and humans is

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bartonella prevalence, no. positive/no. tested (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>p value in model</th>
<th>LRT p value‡</th>
<th>Final model† Adjusted OR (95% CI)</th>
<th>p value in model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>Rats caught before the intervention in all blocks</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Rats caught after the intervention in control blocks</td>
<td>2.16 (0.67–7.39)</td>
<td>0.47</td>
<td>&lt;0.01</td>
<td>2.68 (1.22–6.67)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Rats caught after the intervention in flanking blocks</td>
<td>0.56 (0.18–1.46)</td>
<td>0.26</td>
<td>NA</td>
<td>7.26 (1.56–38.17)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Rats caught after the intervention in intervention blocks</td>
<td>0.12 (0.02–0.46)</td>
<td>&lt;0.01</td>
<td>NA</td>
<td>2.03 (0.22–15.41)</td>
<td>0.50</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>58/267 (22)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>52/233 (22)</td>
<td>1.32 (0.82–2.14)</td>
<td>0.26</td>
<td>0.26</td>
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</tr>
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<td>Sexual maturity</td>
<td>Juvenile</td>
<td>34/177 (19)</td>
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<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>56/277 (20)</td>
<td>0.98 (0.60–1.63)</td>
<td>0.95</td>
<td>0.95</td>
<td>NA</td>
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<tr>
<td>Wound presence</td>
<td>Absent</td>
<td>59/339 (17)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>31/115 (27)</td>
<td>1.67 (0.97–2.81)</td>
<td>0.06</td>
<td>0.06</td>
<td>1.49 (0.83–2.63)</td>
</tr>
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<td>Weight§</td>
<td>NA</td>
<td>1.04 (0.81–1.32)</td>
<td>NA</td>
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<tr>
<td>Presence of fleas on rats</td>
<td>Absent</td>
<td>46/261 (18)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>44/193 (23)</td>
<td>1.39 (0.86–2.25)</td>
<td>0.18</td>
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<tr>
<td>No. fleas on rat</td>
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<td>1.02 (0.95–1.09)</td>
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<td>0.52</td>
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<tr>
<td>Flea index#</td>
<td>NA</td>
<td>1.13 (0.90–1.43)</td>
<td>NA</td>
<td>0.31</td>
<td>0.32</td>
<td>NA</td>
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<tr>
<td>Presence of positive fleas per rat</td>
<td>Absent</td>
<td>67/376 (18)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>23/78 (30)</td>
<td>1.83 (1.00–3.25)</td>
<td>0.04</td>
<td>0.05</td>
<td>1.94 (1.00–3.69)</td>
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<tr>
<td>Season</td>
<td>Summer, June–August</td>
<td>16/124 (13)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td></td>
<td>Fall, September–November</td>
<td>65/208 (31)</td>
<td>3.16 (1.59–6.73)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>2.90 (1.32–6.31)</td>
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<tr>
<td></td>
<td>Winter, December–March</td>
<td>9/122 (7)</td>
<td>0.50 (0.18–1.30)</td>
<td>0.15</td>
<td>NA</td>
<td>0.16 (0.03–0.68)</td>
</tr>
</tbody>
</table>

*OR refers to the odds of Bartonella spp. carriage among rats in each group relative to the reference group for that variable. Variables were included in the final model if they confounded the relationship between the intervention and the outcome (changed the effect of any level of the intervention by ≥10% and/or were associated with the outcome and intervention; p<0.25) or if they were independent predictors that improved the model as indicated by a significant (p<0.05 likelihood ratio test with all confounders and intervention present). LRT, likelihood ratio test; NA, not applicable; OR, odds ratio.
†Final multivariable model: Bartonella status = intervention + wound presence + presence of positive fleas per rat + season + (city.block).
‡Likelihood ratio test comparing the generalized linear mixed model with and without the indicated variable; p<0.05 indicates that the variable significantly improved the model with all confounders and as such was a significant predictor and was retained in the final model.
§Scaled and centered around its mean.
*Average number of fleas per rat per city block.
complex (13). For example, although a previous study revealed that residents in this neighborhood had been exposed to *Bartonella* spp. (3), it is unclear whether this exposure was associated with rats and to what extent humans encounter fleas. Furthermore, for other fleaborne pathogens such as *Yersinia pestis* (agent of the plague), culling rats may increase disease transmission to humans as fleas seek new hosts (14). Understanding how rat abundance and rat removal impacts interspecies and interspecies dynamics and pathogen prevalence is necessary to anticipate management impacts on pathogen transmission.

Whereas our intervention involved removing rats and their fleas, we did not observe a change in the number of fleas on rats. The steady number suggests that culling did not reduce flea abundance, perhaps because *N. fasciatus* fleas also reside in the burrows, such that the number of fleas per rat does not reflect the total number of fleas in a city block (12). It is possible that our intervention removed a negligible proportion of the flea population. In addition, we did not observe a change in the odds of *Bartonella* spp. carriage among fleas. A past study in this neighborhood showed that *Bartonella* spp. carriage among rats was not related to flea presence or abundance; therefore, the role of *N. fasciatus* fleas in the ecology of *Bartonella* spp. in this ecosystem remains enigmatic (15).

Our findings counter a study of *Leptospira interrogans* using the same experimental design, in which culling was associated with an increased odds of infection among rats (5). This difference is likely attributable to differences in transmission; *L. interrogans* is spread via urine (13) and *Bartonella* spp. via fleas (1). Culling may alter a variety of social interactions (e.g., fighting, nest-sharing, grooming) which affect the spread of these pathogens differently. Together, these studies illustrate the complexity of managing rat-associated zoonoses; the intervention may have opposite effects on different pathogens. Indeed, past literature has shown that culling wildlife to control zoonoses can have unpredictable consequences (6) and that ecosystem-based approaches that manage the human–wildlife interface may be more effective.

Acknowledgments

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About the Author

Dr. Byers is the deputy director of the British Columbia Node of the Canadian Wildlife Health Cooperative and a university research associate at Simon Fraser University. At time this research was conducted, she was a PhD student at the University of British Columbia. Her research focus is using systems approaches to derive actionable solutions to One Health issues affecting wildlife, people, and the environment.

References

etymologia

Dermatophilus congolensis [dur′ma-tof′i-las con-gō-len′sis]

Rüdiger D. Ollhoff, Fabio C. Pogliani, Fábio P. Sellera

Dermatophilus congolensis From the Greek derma (skin) + philos (loving), Dermatophilus congolensis is a Gram-positive, aerobic actinomycete, and facultatively anaerobic bacteria. D. congolensis infects the epidermis and produces exudative dermatitis termed dermatophilosis that was previously known as rain rot, rain scald, streptotrichosis, and mycotic dermatitis.

In 1915, René Van Saceghem, a Belgian military veterinarian stationed at a veterinary laboratory in the former Belgian Congo (thus, the species name congolensis), reported D. congolensis from exudative dermatitis in cattle. Local breeders and veterinarians had observed the disease since 1910, but the causal agent was not identified.

Dermatophilosis affects animals, mainly cattle, and more rarely humans. Outbreaks of D. congolensis infection have severe economic implications in the livestock and leather industries.

Sources

Figure 1. Photomicrograph of Dermatophilus congolensis, showing a Giemsa-stained, gram-positive bacteria. Source: Dr. Jerrold Kaplan, Centers for Disease Control, 1965.

Culling of Urban Norway Rats and Carriage of *Bartonella* spp. Bacteria, Vancouver, British Columbia, Canada

Appendix

Methodology

Trapping

Trapping was conducted in the Downtown Eastside neighborhood of Vancouver, Canada from June 2016–January 2017. The study included 12 study sites, each consisting of 3 city blocks (total = 36); 5 were designated as intervention sites and 7 were designated as controls. In intervention sites, lethal trapping occurred in the central block, while the 2 adjacent blocks were considered non-lethal flanking blocks. This designation was meant to account for potential rat movement between contiguous alleyways that might occur in response to the intervention. In control sites, no lethal trapping occurred.

To capture rats, we placed 10 Tomahawk rigid traps (Tomahawk Live Traps, Hazelhurst, WI, USA) in the alley of each city block. Traps were covered with stainless steel covers (Integrated Pest Supplies Ltd, New Westminster, BC, Canada) to prevent vandalism and to minimize contact between captured rats and humans. Traps were chained to immovable objects in alleyways to prevent removal.

Three study sites were trapped at a time, including a total of 9 city blocks and 90 active traps. Prior to active trapping, we prebaited traps for 1 week by baiting and fixing traps open to acclimatize rats to traps and to increase the likelihood of rats entering traps during the active trapping period (1,2). Traps were baited with a peanut butter–oat mixture and Hydrogel (ClearH2O, Westbrook, ME, USA) to provide a water source.

During active trapping periods, traps were set at 4 PM and checked every morning before 7 AM. Traps were active 5 days a week and on the sixth and seventh days, traps were fixed open.
and baited to reacclimatize rats to traps (2). To minimize the potential of pathogen spread among rats due to contamination, all traps were sanitized in 10% bleach after coming into contact with any rat and after any prebaiting period (3).

Active trapping occurred for a total of 6 weeks, divided into 2-week trapping periods. These trapping periods were designated as before; during; or after the intervention. During the before and after periods, all rats that were caught were released at their site of capture after sampling (detailed below). During the intervention period, rats caught in intervention blocks were euthanized by intracardiac injection with pentobarbital after sampling.

**Rat Sampling**

Trapped rats were transported to a mobile laboratory-van where they were covered with a blanket to minimize stress before sampling. Each rat was transferred into an inhalation induction chamber (Kent Scientific, Torrington, CT, USA) and anesthetized with 5% isoflurane in oxygen using an isoflurane vaporizer (Associated Respiratory Veterinary Services, Lacombe, AB, Canada). Anesthesia was maintained throughout sampling through a nose cone administering the anesthetic.

To identify rats which had been previously caught, each rat was given a unique laser-etched ear-tag (Kent Scientific). The following demographic characteristics were recorded for each rat: bodyweight (grams); sexual maturity (maturity was determined as males with scrotal testes and females with a perforate vagina); sex (male or female); and the presence/absence of bite wounds. Bite wounds were considered an important characteristic in this study because they might indicate close contact among individuals (which would allow for fleas to spread among rats) and because *Bartonella* spp. is spread in flea feces and the bacteria is introduced through openings in the skin (4).

Blood was collected from each rat via the jugular and then stored in heparin-coated microtainers (BC, Mississauga, Canada). Fleas were collected from rats in 2 ways. First, the majority of fleas vacated the rat while in the induction chamber and were collected from the induction chamber using tweezers. Second, the coat of each rat was brushed thoroughly over a collection bowl to dislodge any remaining fleas.

Prior to release at their location of capture, rats were allowed to fully recover from the anesthesia (≈15–30 minutes). Individuals that were recaptured were resampled if they were
caught more than 7 days after their prior sampling. This period was informed by guidelines from the University of British Columbia’s Animal Care Committee to ensure that rats had sufficient time to recover between blood collection periods.

**DNA Extraction**

Rat blood and fleas were stored at −80°C before DNA extraction. Fleas were identified to species by observing them under a compound microscope at 40× (5). To extract DNA from fleas, we pooled up to 5 fleas per rat. Flea pools were surface-sterilized in 10% bleach and then rinsed in nuclease-free water and twice in 100% ethanol to remove bacteria from the external body. Flea pools were then crushed using a sterile scalpel. DNA from rat blood and fleas was extracted using the QIAgen DNEasy Blood and Tissues Kit following the manufacturer’s protocol.

Detailed methods for *Bartonella* spp. testing are outlined in Himsworth et al. (6).

**Statistical Analysis**

**Model A** The impact of the intervention on *Bartonella* spp. carriage among rats.

To assess the impact of the intervention on *Bartonella* spp. carriage among rats we built mixed effects logistic regression models, otherwise known as generalized linear mixed models, or GLMMs. The outcome for Model A was the *Bartonella* spp. status of individual rats (positive or negative). The intervention was expressed statistically as a categorical variable consisting of four levels and was an indicator of whether rats were captured: 1) in the 2 weeks before the intervention in any block type (the reference category); 2) in the 2-week period after the intervention in control blocks; 3) in the 2-week period after the intervention in flanking blocks; or 4) in the 2-week period after the intervention in intervention blocks. Although we did not perform the intervention in control and flanking blocks, we considered the 2-week trapping periods after the intervention in control and flanking blocks independently to detect temporal changes in prevalence not related to the intervention. Finally, we included an indicator of the city-block as a random effect in all steps of the modeling process to control for heterogenous prevalence of *Bartonella* spp. among blocks (7).

For each GLMM we took a hypothesis testing model building approach to estimate the effect of the intervention on the *Bartonella* spp. status of each rat while controlling for important covariates. Potential covariates included sex (male or female), sexual maturity (juvenile or mature), weight (g), presence of fleas (present or absent), presence of bite wounds (present or
absent), number of fleas (count), flea index (average number of fleas per rat in a city-block),
presence of positive fleas (present or absent), and season (summer, fall, winter). Summer was
designated as June–August, fall was September–November, winter was December–March.

We included covariates in the model that were either confounders of the relationship
between the intervention variable and the outcome or that were independent significant
predictors of the outcome. In step one, we assessed whether covariates were confounders. To do
this, we first examined the relationships between each potential confounder and the outcome in
separate GLMMs containing only that variable and the random effect of the city block. Variables
associated with the outcome (p<0.25) were then assessed for their impact on the intervention
variable. To do this, we evaluated whether the inclusion of each covariate, that was associated
with the outcome (p<0.25), in a GLMM containing that variable and the intervention variable,
changed the effect estimate of any level of the intervention (i.e., rats caught before the
intervention in any block type, rats caught after the intervention in control blocks, etc.) by ≥10%
relative to its effect estimate in a GLMM containing only the intervention variable. If the
variable met both of these criteria, they were considered confounders. In step two, variables that
did not meet the confounder criteria, but were still significantly (p<0.05) associated with the
outcome were considered as independent significant predictors. Each of these variables were
entered into the model containing the intervention and identified confounders. Variables were
retained in the model if they substantially decreased the standard error of any level of the
intervention variable effect estimates and/or if they significantly improved the model as
evidenced by a significant likelihood ratio test (p<0.05) that compared the overall model with
and without that variable. The final model contained all confounders and significant predictors
that met these criteria.

Thirty-three animals were recaptured; 13 were recaptured in the 2 weeks before the
intervention and 20 were recaptured after. For rats that were recaptured within the same period
(before or after) rat characteristics were either averaged (e.g., average weight) or for
characteristics that were categories (i.e., juvenile vs. mature) the value for the latest capture was
used. Further, if any of these recaptured rats tested positive once, then they were considered to be
*Bartonella* spp. positive. The resulting dataset included 454 rats.
Model B The impact of the intervention on Bartonella spp. carriage among fleas.

The impact of the intervention on Bartonella spp. carriage by fleas was modeled following the same GLMM building process as for rats. However, in this model the outcome was the Bartonella spp. status of flea pools (positive or negative), and the intervention variable categorized whether fleas were taken from rats before the intervention in any block type, after the intervention in control blocks, after the intervention in flanking blocks, or after the intervention in intervention blocks.

Among 33 recaptured rats, 7 had fleas that were tested. If these rats were captured in the same 2-week trapping period, the average number of fleas collected across the captures was used and the fleas were considered positive from that rat if they tested positive in ≥1 recapture in that 2-week period. The dataset included 201 rats from which fleas were collected.

Model C The impact of the intervention on the number of fleas on rats.

To assess whether the intervention influenced the number of fleas counted on each rat, we used a negative binomial GLMM to account for overdispersion in the outcome. The outcome was the number of fleas counted on each rat. This model was built following the same procedure as for Models A and B. This analysis used the same 454 rats as Model A.

References: