Title

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Journal

Journal of Feline Medicine and Surgery, 19(8)

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Publication Date

2017-08-01

DOI

10.1177/1098612X16658317

Peer reviewed

Original Article

Journal of Feline Medicine and Surgery
2017, Vol. 19(8) 798–802

DOI: 10.1177/1098612X16658317
journals.sagepub.com/home/jfms

This paper was handled and processed by the American Editorial Office (AAFP) for publication in *JFMS*

Assessment of the ability of *Aedes* species mosquitoes to transmit feline *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum'

Krystle L Reagan*, Lorelei L Clarke, Jennifer R Hawley, Phillip Lin and Michael R Lappin

Abstract

Objectives The objective of this study was to evaluate wild-caught mosquitoes for evidence of hemotropic *Mycoplasma* species DNA and to determine whether the feline hemoplasmas, *Mycoplasma haemofelis* (Mhf) and '*Candidatus* Mycoplasma haemominutum' (Mhm), can be transmitted by *Aedes aegypti* mosquitoes in a laboratory setting.

Methods Wild-caught mosquito pools (50 mosquitoes per pool, 84 pools) utilized in routine public health department disease surveillance programs were tested for hemotropic *Mycoplasma* species DNA using PCR with primers designed to amplify all known hemoplasmas. Additionally, mosquitoes were trapped in the vicinity of known feral cat colonies, pooled (50 mosquitoes per pool) and tested (84 pools). Purpose-bred cats housed in a research facility were infected with Mhf or Mhm and then colonized laboratory *A aegypti* were fed upon the bacteremic cats. After a 7 day incubation period, mosquitoes previously fed on infected cats were allowed to feed again on naive cats, which were monitored for bacteremia for 10 weeks.

Results *Mycoplasma wenyonii* DNA was confirmed in one wild-caught mosquito pool by DNA sequencing. While 7% of cats tested in feral colonies were hemoplasma positive, none of the mosquitoes trapped near colonies were positive. Hemoplasma DNA was amplified from *A aegypti* by PCR immediately after the infectious blood meal, but DNA was not detected at 7 and 14 days after feeding. Although evidence for uptake of organisms existed, hemoplasma DNA was not amplified from the experimentally infested cats in the 10 week observation period.

Conclusions and relevance While wild-caught mosquitoes contained hemoplasma DNA and laboratory reared *A aegypti* mosquitoes take up hemoplasmas during the blood meal, there was no evidence of biologic transmission in this model.

Accepted: 13 June 2016

Introduction

Mycoplasma haemofelis (Mhf; previously *Haemobartonella felis*), '*Candidatus* Mycoplasma haemominutum' (Mhm) and '*Candidatus* Mycoplasma turicensis' (Mtc) are the most common hemotropic *Mycoplasma* species (hemoplasmas) in cats and are recognized around the world.[1–3] Cats with hemoplasmosis have clinical abnormalities that vary from subclinical infection to waxing and waning lethargy, anorexia, and fever to a potentially fatal hemolytic state. Cross protection amongst species does not occur and cats can be infected with one or more of these organisms. In one experimental study, cats infected with Mhm followed by Mhf had more significant clinical illness than cats infected with either organism alone.[4] Dual infection with Mhm and Mhf or coinfection with feline immunodeficiency virus (FIV) or feline leukemia

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virus (FeLV) can worsen signs of clinical disease.[5] Many cats survive the initial stage of infection and become chronic carriers without ongoing clinical signs.[2]

As the hemoplasmas are erythrocyte-associated, *Ctenocephalides felis* has been tested for presence of organisms. Amplification of Mhf and Mhm DNA has been identified from *C felis* isolated from cats in several countries.[6–8] It has been hypothesized that fleas are primarily responsible for transmission among felids. However, the distribution of the feline hemoplasmas extends beyond the distribution of *C felis* with infection rates of 12–14% in non-flea-endemic areas such as Colorado, USA.[9,10] An alternate vector may, in part, explain high hemoplasma prevalence rates in regions without *C felis*. Mosquitoes are found nationwide in the USA and have shown an ability to transmit other blood-borne parasites to cats.[11] The objectives of this study were to explore the potential that feline hemoplasmas could be transmitted by mosquitoes using wild-caught mosquitoes and laboratory reared *Aedes aegypti* in an experimental model.

Materials and methods

Experiment 1

Mosquitoes caught with a CO_2-baited CDC light trap during a West Nile virus surveillance study in Fort Collins, Colorado (CO), during June, July and August of 2006 and 2007 were utilized for this study. The mosquitoes were collected from traps in a park, by a fish hatchery, in a natural area and adjacent to a reservoir. *Aedes vexans* were identified using anatomic markers and pooled into samples of 50 mosquitoes each.

Experiment 2

The CO_2-baited CDC light traps were placed near known feral cat colonies around Fort Collins, CO, during June and August 2009. The mosquitoes were grouped into *A vexans, Culex tarsalis* and other species pools of 50 mosquitoes each. Cats (n = 81) in the area of the mosquito traps were captured during an ongoing trap–spay–neuter program and 1 ml blood in EDTA was collected by venepuncture as part of FeLV and FIV testing. Remnant blood was transported on cold packs and refrigerated until processing for hemoplasma PCR assay.

Experiment 3

A total of four mixed sex, specific pathogen-free, 6-month-old cats were used with institutional animal care and use approval. To initiate hemoplasma infections, one cat was inoculated intravenously (IV) with 0.5 ml blood collected from a cat previously infected a wild-type strain of Mhm. One cat was inoculated IV with 0.5 ml blood collected from a cat a naturally infected with Mhf. Over the course of the experiment, all four cats were monitored daily for any signs of clinical illness such as lethargy or anorexia.

Laboratory reared *A aegypti* RexD strain raised at the Arthropod-borne Infectious Disease Laboratory (Fort Collins, CO) were used to feed on the experimentally infected cats. The mosquitoes were housed at insectary conditions at 80% humidity and 80°F with a 12 h:12 h light:dark cycle (Figure 1). After the IV-inoculated cats were confirmed by PCR assay to be positive for the respective inoculated hemoplasma, they were sedated with dexmedetomidine 0.01 mg/kg, butorphanol 0.2 mg/kg and ketamine 2 mg/kg intramuscularly and placed into individual chambers with 200 female *A aegypti* which were allowed to feed for 30 mins. At the end of the feeding period, two mosquitoes from each chamber were removed, pooled and assessed for hemoplasma DNA using PCR assays. The remaining blood-fed mosquitoes were housed under insectary conditions for 7 days with water and sugar provided ad libitum. At that time, the two naïve cats were sedated as per the protocol outlined above, placed in individual chambers, and the appropriate *A aegypti* groups (either Mhf or Mhm fed) were allowed to feed on the cats for 30 mins (Figure 2). Venepuncture was used to obtain 0.5–1.0 ml of blood from the cats weekly for 10 weeks for performance of the hemoplasma PCR assay.

Experiment 4

After the feeding period in experiment 3 of the female adult, *A aegypti* were evaluated for the presence of Mhf or Mhm DNA. The mosquitoes were cold anesthetized at –20°C for approximately 1 min and sorted on ice. Legs and wings were removed from each mosquito and discarded. The proboscis was then placed into a 10 µl glass capillary tube filled with immersion oil type B and the mosquitoes were left to salivate in insectary conditions for 2 h. Oil and saliva were collected from capillary tubes into a 1.7 ml tube with 500 µl Dulbecco's modified Eagle's medium (unsupplemented) as previously described.[12]

Figure 1 Mosquito cages. *Aedes aegypti* mosquitoes were housed in cages under insectary conditions. Cats were sedated and introduced into the cages for feeding experiments

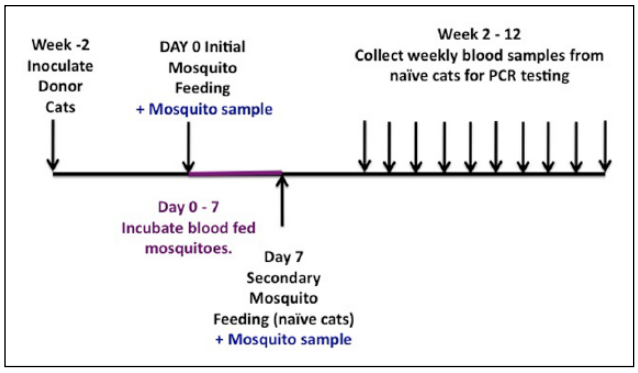

Figure 2 Timeline for experiments 3 and 4

The mixture was vortexed then centrifuged in a tabletop centrifuge at 12,000 rpm (9000 *g*) for 10 mins. Using a 28 G needle and a 1 ml syringe, the aqueous layer was removed taking care not to include any oil. DNA was extracted and a hemoplasma PCR assay performed.

Assays

Extraction of total DNA from all mosquitoes used in the experiments was undertaken using a modified QIAGEN DNeasy Blood and Tissue kit. Mosquito pools were poured into tubes along with three copperhead BBs and 2 ml sterile phosphate buffered saline (PBS). The mosquitoes were then vortexed for 30–60 s and centrifuged at 4°C for 11 mins at 34,000 rpm (13000 g). At total of 180 µl supernatant was extracted from the tubes and purified using the 'purification of total DNA from insects' protocol included in the kit. Total DNA was extracted from each feline blood sample (200 µl) using a DNA extraction robot (Corbett Robotic) and a commercially available kit (QIAGEN). DNA from each sample was then quantified using a Nanodrop (ThermoScientific).

All DNA extracts (cats and mosquitoes) were evaluate for hemoplasma DNA using a previously described protocol.[10] Positive amplicons were sequenced at a core facility (Macromolecular Resources, Colorado State University) with sequencing used to confirm the presence of hemoplasma DNA. A composite Mhf- and Mhm-positive and negative samples were included as controls in each PCR assay.

To determine whether the trapped mosquitoes in experiments 1 and 2 contained mammalian blood, a cytochrome B PCR assay was performed as previously described using the following modifications.[13] DNA amplification was performed in a 25 µl reaction mixture containing 1 ng template DNA, 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP and dUTP, 0.5 µM of each primer and 1.25 units of AmpliTaq Gold DNA Polymerase (Applied Biosystems). Amplifications were performed in an automated thermal cycler using a time-release PCR assay protocol as follows: 95 ºC for 10 mins, 35 cycles of amplification at 94 ºC for 1 min, 50 ºC for 1 min and 72 ºC for 2 mins, followed by postincubation at 72 ºC for 10 mins. PCR amplification products were identified by ethidium bromide fluorescence after electrophoresis in 1.5% agarose gels, which were then photographed. Hemoplasma DNA extracted from cat blood was used as a positive control.

Results

Experiment 1

A total of five of the 84 *A vexans* pools were positive in the hemoplasma PCR assay. Sequencing confirmed one of the samples to be *M wenyonii* and the other four samples were *Spiroplasma* species. Cytochrome B DNA was amplified from 2/84 (2.4%) of the *A vexan* pools; the pool was not positive for *M wenyonii* DNA.

Experiment 2

Of the 81 feral cats, five (6.2%) were confirmed by sequencing to harbor Mhf (two cats), Mhm (two cats) or both (one cat). The mosquito traps that were set up in proximity of known feral cat colonies yielded 21 pools of *A vexans*, 30 pools of *C tarsalis* and 25 pools of other species. None of these pools were PCR positive for either cytochrome B or hemoplasma DNA.

Experiment 3

The *A aegypti* collected from the two chambers immediately after being allowed to feed on the Mhf or Mfm IV inoculated cats were positive for the appropriate hemoplasma DNA. However, none of the blood samples collected from the cats after the previously fed *A aeqypti* were allowed to feed a second time after the 7 day incubation period were positive for hemoplasma DNA.

Experiment 4

On days 7 and 14 after feeding on Mhf- or Mhm-infected cats, DNA extracted and pooled from five whole female *A aegypti* and from extracted saliva were negative for hemoplasma DNA.

Discussion

Amplification of *M wenyonii* DNA from one *A vexans* pool in experiment 1 documents that the mosquitoes had fed on a large animal,[14] and that uptake of hemoplasmas can occur as mosquitoes are taking a blood meal from a bacteremic animal. Uptake of Mhf and Mhm by *A aegypti* was also documented in experiment 3 of this study. The other four positive amplicons from wild-caught *A vexans* in experiment 1 were *Spiroplasma* species, which are organisms that infect insects, not mammals. This finding emphasizes that genetic sequencing results should be used as the gold standard for identification of infectious agents when broad-range primer designs are used.

The hemoplasma prevalence rate from the feral cats was similar but slightly lower than previously reported in the region.[3,10] However, the results still confirm the presence of the two most common feline hemoplasmas, Mhm and Mhf, in the area. While *A vexans* and *C tarsalis* were commonly found in the area and are known to feed on cats,[11] none of the pools tested were positive for feline hemoplasmas. These mosquitoes were also negative for cytochrome B, which suggests they had not recently fed on mammalian hosts. The method of trapping utilized in this study typically attracts mosquitoes seeking a blood meal, not those that have recently fed.[15] Further studies using methods that target recently blood-fed mosquitoes could further determine if feline hemoplasmas are taken up during the blood meal in the wild.

While both Mhm and Mhf were shown to be taken up during the blood meal by *A aegypti* in experiment 3, neither of the cats that were subsequently exposed to the previously fed (7 days prior) mosquitoes became positive for either hemoplasma. In addition, Mhm or Mhf DNA was not amplified from the previously fed (7 and 14 days) *A aegypti* whole mosquitoes or saliva in experiment 4. These results suggest that Mhm and Mhf do not colonize *A aegypti* and that this mosquito is not a biological vector for these hemoplasmas. However, the results described herein cannot exclude the possibility that *A aegypti* could be a mechanical vector as only one re-feeding time point was used. In future experiments, interrupted feeding should be used to assess for evidence for mechanical transmission by mouthparts.

To further assess mosquitoes' role in potential transmission of Mhf or Mhm, laboratory experiments were conducted with laboratory adapted mosquitoes. A mosquito species of the same genus was chosen because no established laboratory colonies of *A vexans* were available. *A aegypti* is a known vector for many viruses, including yellow fever virus and dengue virus. It cannot be ruled out from this study that the mosquitoes would have tested positive at alternate points than those tested. Vector–host relationships can be very restricted from some disease life cycles, and alternate outcomes may be obtained had another species of mosquito been used in this experiment.

Conclusions

These studies indicate that mosquitoes are likely not a primary method of transmission for feline hemoplasmal organisms, but could potentially act as mechanical vectors since they are, in fact, capable of taking up the organisms in a blood meal. Our field data suggest that if such transmission events occur naturally, they are rare in the ecosystem analyzed. Further studies investigating other mosquito species and the potential of mechanical transmission may better define the potential of mosquitoes to act as vectors in the field.

These data allow for future studies to investigate non-vectored modes of transmission to explain the distribution pattern of the feline hemoplasmas.

Acknowledgements The authors acknowledge Dr Lars Eisen for training and loaning the mosquito traps and providing us with the mosquito pools.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding The study was funded by a Young Investigator Grant from the Center for Companion Animal Studies at Colorado State University.

References

1 Willi B, Boretti FS, Baumgartner C, et al. **Prevalence, risk factor analysis, and follow-up of infections caused by three feline hemoplasma species in cats in Switzerland**. *J Clin Microbiol* 2006; 44: 961–969.

2 Ishak AM, Radecki S and Lappin MR. **Prevalence of *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', *Bartonella* species, *Ehrlichia* species, and *Anaplasma phagocytophilum* DNA in the blood of cats with anemia**. *J Feline Med Surg* 2007; 9: 1–7.

3 Sykes JE, Terry JC, Lindsay LL, et al. **Prevalences of various hemoplasma species among cats in the United States with possible hemoplasmosis**. *J Am Vet Med Assoc* 2008; 232: 372–379.

4 Westfall DS, Jensen WA, Reagan WJ, et al. **Inoculation of two genotypes of *Hemobartonella felis* (California and Ohio variants) to induce infection in cats and the response to treatment with azithromycin**. *Am J Vet Res* 2001; 62: 687–691.

5 George JW, Rideout B, Griffey SM, et al. **Effect of pre-existing FeLV infection or FeLV and feline immunodeficiency virus coinfection on pathogenicity of the small variant of *Haemobartonella felis* in cats**. *Am J Vet Res* 2002; 63: 1172–1178.

6 Barrs VR, Beatty JA, Wilson BJ, et al. **Prevalence of *Bartonella* species, *Rickettsia felis*, haemoplasmas and the *Ehrlichia* group in the blood of cats and fleas in eastern Australia**. *Aust Vet J* 2010; 88: 160–165.

7 Lappin MR, Griffin B, Brunt J, et al. **Prevalence of *Bartonella* species, haemoplasma species, *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Neorickettsia risticii* DNA in the blood of cats and their fleas in the United States**. *J Feline Med Surg* 2006; 8: 85–90.

8 Shaw SE, Kenny MJ, Tasker S, et al. **Pathogen carriage by the cat flea *Ctenocephalides felis* (Bouché) in the United Kingdom**. *Vet Microbiol* 2004; 102: 183–188.

9 Hackett TB, Jensen Wa, Lehman TL, et al. **Prevalence of DNA of *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum,' *Anaplasma phagocytophilum*, and species of *Bartonella*, *Neorickettsia*, and *Ehrlichia* in cats used as blood donors in the United States**. *J Am Vet Med Assoc* 2006; 229: 700–705.

10 Jensen WA, Lappin MR, Kamkar S, et al. **Use of a polymerase chain reaction assay to detect and differentiate**

two strains of *Haemobartonella felis* in naturally infected cats. *Am J Vet Res* 2001; 62: 604–608.

11 Gomes LAM, Serrão ML, Duarte R, et al. **Attraction of mosquitoes to domestic cats in a heartworm enzootic region**. *J Feline Med Surg* 2007; 9: 309–312.

12 Colton L, Biggerstaff BJ, Johnson A, et al. **Quantification of West Nile virus in vector mosquito saliva**. *J Am Mosq Control Assoc* 2005; 21: 49–53.

13 Nakaki S-i, Hino D, Miyoshi M, et al. **Study of animal species (human, dog and cat) identification using a multiplex single-base primer extension reaction in the cytochrome b gene**. *Forensic Sci Int* 2007; 173: 97–102.

14 Song Q, Wang L, Fang R, et al. **Detection of *Mycoplasma wenyonii* in cattle and transmission vectors by the loop-mediated isothermal amplification (LAMP) assay**. *Trop Anim Health Prod* 2012; 45: 247–250.

15 Chen Y-C, Wang C-Y, Teng H-J, et al. **Comparison of the efficacy of CO_2-baited and unbaited light traps, gravid traps, backpack aspirators, and sweep net collections for sampling mosquitoes infected with Japanese encephalitis virus**. *J Vector Ecol* 2011; 36: 68–74.