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CASE REPORT

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Evidence for vertical transmission of *Mycoplasma haemocanis*, but not *Ehrlichia ewingii*, in a dog

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Abstract

A 2-year-old female intact pregnant Beagle was evaluated after the owner surrendered her to a shelter. Prepartum and 2 months postpartum at the time of routine spay, the dam was whole-blood polymerase chain reaction (PCR) positive for *Ehrlichia ewingii*. She was also whole-blood PCR positive for *Mycoplasma haemocanis* prepartum and continuously for 5 months thereafter. The dam delivered 5 healthy puppies, 1 of which was whole-blood PCR positive for *M. haemocanis*. All 5 puppies had antibodies against *Ehrlichia* spp. at 1 month of age but not thereafter, and all puppies were *Ehrlichia* spp. PCR negative for 5 months of follow-up. Therefore, this study supports a potential role for vertical transmission in the maintenance of *M. haemocanis* in dogs as reservoir hosts. In contrast, in this case there was no evidence that *E. ewingii* was transmitted transplacentally or during the perinatal period.

KEYWORDS
bacterial species, hemotropic mycoplasma, perinatal, rickettsia, vector-borne

1 | INTRODUCTION

Canine hemotropic *Mycoplasma* species, including *Mycoplasma haemocanis* and "*Candidatus* Mycoplasma haematoparvum," are epicellular erythrocytic bacteria thought to cause infectious hemolytic anemia, most often reported in immunocompromised, splenectomized, or coinfected dogs.[1-5] Both of these canine hemotropic *Mycoplasma* species are possible zoonoses.[6-8] Transmission between dogs is suspected to be mainly vector-borne,[2,9] but a role for vertical transmission has also been proposed.[1,10,11]

Several *Ehrlichia* spp. infect dogs in the United States, including *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, Panola Mountain *Ehrlichia* spp., and *Ehrlichia muris*. *Ehrlichia ewingii*, an organism found most often in neutrophils, is the most prevalent *Ehrlichia* species serologically detected in dogs in the southern United States and is associated with fever, anorexia, thrombocytopenia, polyarthritis, and central nervous system abnormalities.[12,13] *Ehrlichia ewingii* is transmitted by *Amblyomma americanum*, the lone star tick. After tick transmission, dogs can remain infected for over 2 years.[13] Dogs are an important reservoir host and could be a source of zoonotic *E. ewingii* infections.[14,15]

Vertical transmission has been proposed as a secondary route of exposure for several canine vector-borne diseases (CVBDs) including cyclic canine thrombocytopenia (*Anaplasma platys*),[16,17] Lyme borreliosis (*Borrelia burgdorferi*),[18] hepatozoonosis (*Hepatozoon canis*),[19] leishmaniosis (*Leishmania infantum*),[20] and Chagas disease (*Trypanosoma cruzi*).[21] Vertical transmission from dam to puppies was not found to occur for *Anaplasma phagocytophilum*, based on a single case report,[22] but it has been reported in 1 human case and in naturally and experimentally infected cows.[23-25] Diagnostic support for vertical transmission

Abbreviations: CVBD, canine vector-borne disease; IFA, immunofluorescent antibody; PCR, polymerase chain reaction.

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of *Ehrlichia canis* was not found in 12 infected or exposed dams and their puppies,[26] although that single study does not definitively rule out the possibility that transplacental transmission occurs. The potential role for vertical transmission in many other CVBDs, including hemotropic *Mycoplasma* spp. and *E. ewingii*, remains unreported or incompletely described.

This case report describes sequential microbiological results for a pregnant dog naturally infected with *E. ewingii* and *M. haemocanis*, and postpartum results for her puppies. Our findings support vertical transmission of *M. haemocanis*, but not *E. ewingii* from the dam to her puppies.

2 | CASE HISTORY AND DIAGNOSTIC TESTING

A 2-year-old female intact Beagle was evaluated after the owner surrendered her to a shelter in eastern North Carolina. At the time of surrender, the dog had a severe flea and tick infestation and was determined to be pregnant. No medical history information was available. The owner of a veterinary clinic in Raleigh, North Carolina assumed responsibility for, and costs associated with, the care of the dog from the shelter, and the dog was transferred to the veterinary clinic before whelping. At the veterinary clinic, ectoparasites were removed manually followed by an acaricidal bath. Ticks were identified by the attending veterinarian as *Rhipicephalus sanguineus*. Two days later, the dam delivered 6 puppies naturally: 2 males and 4 females. Immediately before and after whelping, the dam and puppies were housed indoors in a room shared with cats but no other dogs. The cats in this room were permanent residents of the veterinary clinic. After initial ectoparasite removal, ectoparasites were not found during daily examinations. Prepartum, the dam received core vaccinations and was dewormed for intestinal parasites. At 6 weeks postpartum, Trifexis (Elanco, Greenfield, Indiana) was administered PO on a monthly basis for flea, heartworm, and helminth prevention. After weaning (6 weeks postpartum), the dam was adopted and 2 weeks after weaning an ovariohysterectomy was performed. One year later, the dam remained apparently healthy in the care of her owners.

One male puppy (third puppy delivered) failed to nurse, and despite bottle-feeding died within 24 hours of birth. A gross postmortem examination was performed. The remaining 5 puppies appeared to be healthy, and ectoparasites were not found during daily examinations. The puppies received routine preventative care and were housed with the dam in the veterinary clinic until weaning at 6 weeks of age. Starting at 6 weeks of age, the puppies were also treated with Trifexis (Elanco) monthly. After weaning, all 5 puppies were adopted and 1 year later remained apparently healthy in the care of their owners.

Informed consent for sequential diagnostic testing was obtained initially from the shelter and after adoption subsequently from each owner. Sequential diagnostic testing for dam and puppies included immunofluorescent antibody (IFA) assays for *Babesia canis*, *Babesia gibsoni*, *Bartonella henselae*, *Bartonella koehlerae*, *Bartonella vinsonii* subsp. *berkhoffii*, *E. canis*, and *Rickettsia rickettsii* (spotted fever group *Rickettsia*); whole-blood polymerase chain reaction (PCR) assays for *Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., hemotropic *Mycoplasma* spp., and *Rickettsia* spp.; and a commercial ELISA-based assay (SNAP 4Dx Plus, IDEXX Laboratories, Inc, Westbrook, Maine) for *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*), *B. burgdorferi*, and *Ehrlichia* spp. (*E. canis*, *E. chaffeensis*, and *E. ewingii*) antibodies, and *Dirofilaria immitis* antigen. All serum, whole blood, and tissue samples were tested by the North Carolina State University College of Veterinary Medicine Vector Borne Disease Diagnostic Laboratory (Raleigh, North Carolina) using previously described methods.[27-35] Seroreactive samples were defined as having endpoint IFA titers ≥1:64.

Because of her flea and tick infestation, the dam was tested for evidence of vector-borne infections with CVBD serology and PCR assays at the time when responsibility for her care was taken over by the veterinary clinic, 2 days before parturition. A complete blood count and serum chemistry panel was also obtained from the dam at that time. With permission of the owner, follow-up PCR panel and SNAP 4Dx Plus ELISA testing was performed on the dam monthly for 5 months postpartum, and follow-up IFA testing was performed at 5-month time-point. Because of financial constraints and the subsequent apparent health of the dam, sequential complete blood counts, serum chemistry profiles, and urinalyses were not obtained. Placenta from 1 puppy along with uterine and ovarian tissue from the dam at the time of ovariohysterectomy was also tested via the multiple PCR assays described above. Selected tissues retrieved during postmortem examination from the puppy that died within 24 hours of birth (heart blood, mesenteric lymph node, spleen, and bone marrow) were tested using the same PCR assays. With permission from each individual owner, the remaining 5 puppies were tested by whole blood PCR assays and SNAP 4Dx Plus ELISA monthly at 1 to 5 months of age, and IFA panel at 3 and 5 months of age. Because of financial constraints and the apparent health of these puppies, complete blood counts, serum chemistry profiles, and urinalyses were not obtained. The dam and puppies underwent a follow-up examination and were tested by SNAP 4Dx Plus ELISA at approximately 1 year postpartum. The timeline of events and CVBD testing schedule is shown in Figure 1. Test selection and testing intervals were also determined by limitations in obtaining blood specimens from newborn puppies.

3 | RESULTS

Prepartum, DNA extracted from whole blood from the dam was PCR positive for *M. haemocanis*. The dam's blood remained PCR positive at all time-points tested (monthly from 1 to 5 months postpartum; Figure 1). In addition, left uterine and right ovarian tissues obtained after ovariohysterectomy at 2 months postpartum were also *M. haemocanis* PCR positive.

Prepartum, *E. ewingii* DNA was PCR amplified from the dam's blood. At that time, the dam was SNAP 4Dx Plus ELISA positive for *Ehrlichia* spp. antibodies but *E. canis* IFA was negative, reflecting a lack of cross-reactivity between *E. ewingii* and *E. canis*. Because *E. ewingii* has never been successfully isolated in cell culture (as a source of antigen for IFA testing), *E. ewingii* IFA was not possible. The dam's blood

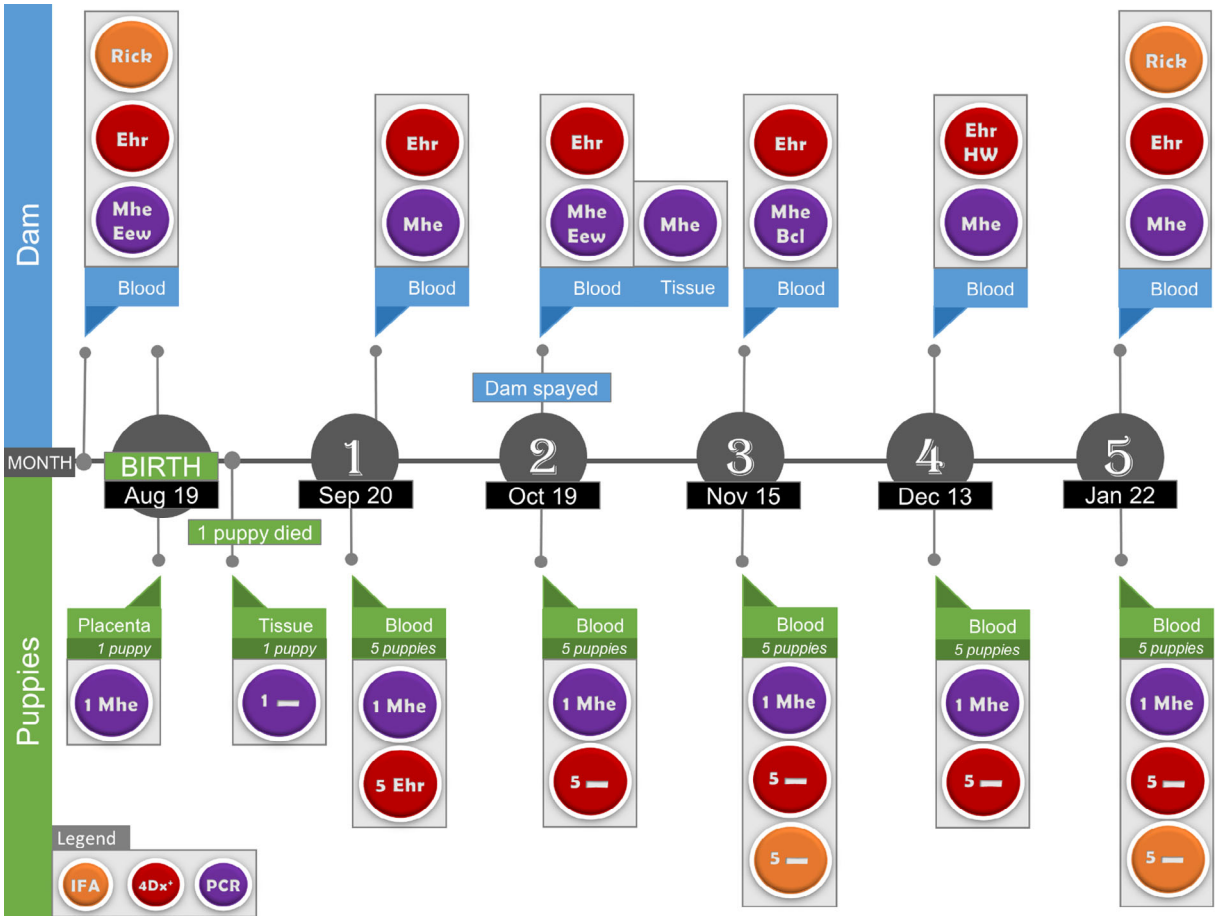

FIGURE 1 Timeline showing the timing of testing, tissue tested, and results from each test for the dam (upper, blue) and puppies (lower, green). One-year follow-up is not included. The number of puppies that had each type of tissue tested at each time-point is also shown (green bar). Each colored circle represents a test that was performed (orange, IFA; red, 4Dx SNAP Plus; purple, PCR). Positive results for each test are shown in text within each circle; circles with a dash (–) indicate negative tests. The number of puppies with each result is indicated within each circle. Bcl, *Bartonella clarridgeiae*; Eew, *Ehrlichia ewingii*; Ehr, *Ehrlichia* spp.; HW, *Dirofilaria immitis*; IFA, immunofluorescent antibody; Mhe, *Mycoplasma haemocanis*; PCR, polymerase chain reaction; Rick, *Rickettsia* spp.

was also *E. ewingii* PCR positive at 2-month postpartum time-point (at the time of ovariohysterectomy), but was PCR negative at the 1 and 3 to 5-month time-points. *Ehrlichia ewingii* was confirmed via species-specific qPCR assay; the dam's blood was PCR negative for *E. canis*, *E. chaffeensis*, and Panola Mountain *Ehrlichia* spp. Uterine and ovary tissues were *Ehrlichia* spp. PCR negative. The dam remained SNAP 4Dx Plus ELISA seroreactive throughout all time-points, and had not seroconverted to *E. canis* antigen by IFA testing at the 5-month time-point. At the time of adoption, the dam's complete blood count and serum chemistry panel were normal with the exception of a mild microcytic non-regenerative anemia (HCT 33.2%) and mild neutrophilia (13.58 K/uL). As no significant clinical signs of ehrlichiosis or hemotropic *Mycoplasma* spp. infection were noted, the dam was *E. ewingii* PCR negative at 4 postpartum testing time-points, and antimicrobial treatment is not consistently recommended for dogs with subclinical *M. haemocanis* infection,[36] antibiotics were not administered.

The dam was *R. rickettsii* IFA seroreactive prepartum (IFA titer 1:64) and again at the 5-month time-point (1:128). She was *Rickettsia* spp. PCR negative at all time-points. *Bartonella clarridgeiae* DNA was PCR amplified and sequenced from the dam's blood at the 3-month time-point, but blood was *Bartonella* PCR negative at all other time-points. The dam was not IFA seroreactive to any *Bartonella* spp. tested in the CVBD comprehensive panel (*B. henselae*, *B. vinsonii* subsp. *berkhoffii*, or *B. koehlerae*) prepartum or at the 5-month time-point. At the 4-month time-point, she was positive for *D. immitis* on the SNAP 4Dx Plus ELISA, but was microfilariae negative and negative on the SNAP 4Dx Plus ELISA again at the 5-month time-point. These findings were confirmed by repeated testing on stored samples. The dam was seronegative for *Anaplasma* spp. and *B. burgdorferi* on the 4Dx SNAP Plus ELISA at all time-points, and was seronegative for *B. canis* and *B. gibsoni* on IFA at both the prepartum and 5-month time-points.

At approximately 1 year postpartum, the dam was again positive for *D. immitis* on the SNAP 4Dx Plus ELISA, but was microfilariae negative. Treatment for heartworm disease based on American Heartworm Society recommendations[37] was initiated at this time. The dam remained positive for *Ehrlichia* spp. antibodies, and remained negative for *Anaplasma* spp. and *B. burgdorferi* antibodies by SNAP 4Dx Plus ELISA at that time.

Tissues from the puppy that died within 24 hours of birth were PCR negative for *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Ehrlichia* spp., hemotropic *Mycoplasma* spp., and *Rickettsia* spp. There were no abnormalities on gross postmortem examination, and histopathology was not performed.

The 5 surviving puppies were monitored for *Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Ehrlichia* spp., hemotropic *Mycoplasma* spp., and *Rickettsia* spp. by whole blood PCR assays monthly from 1 to 5 months of age. One female puppy was *M. haemocanis* PCR positive at 1 month, and remained PCR positive at all 4 subsequent time-points (placenta tissue from this puppy was not available for testing). The 16S rDNA *M. haemocanis* sequence from this puppy matched the sequence obtained from the dam (128/128 base pairs, 100% identity). This puppy was apparently healthy with no clinical signs of hemotropic *Mycoplasma* spp. infection, and was therefore not treated with antibiotics. The other 4 surviving puppies, as well as the puppy who died, were hemotropic *Mycoplasma* spp. whole blood PCR negative at all testing time-points, despite *M. haemocanis* PCR positive placenta tissue from 1 of these female puppies.

The 5 surviving puppies were *Ehrlichia* spp. SNAP 4Dx Plus ELISA seroreactive at 1 month of age, but became seronegative by 2 months and remained negative for all remaining time-points (monthly through 5 months of age). All puppies remained *Ehrlichia* spp. whole blood PCR negative during the 5 month study period. *Ehrlichia ewingii* cross-reactive antibodies to *E. canis* antigens were not detected by IFA at 3 or 5 months of age.

The 5 surviving puppies were *Bartonella* spp. (*B. henselae*, *B. vinsonii* subsp. *berkhoffii*, and *B. koehlerae*) and *R. rickettsii* IFA non-seroreactive at 3 and 5 months of age, and were *Bartonella* spp. and *Rickettsia* spp. PCR negative at all time-points. All 5 puppies were negative for *D. immitis* antigen and *Anaplasma* spp. and *B. burgdorferi* antibodies by SNAP 4Dx Plus ELISA at all time-points. At the time of follow-up at approximately 1 year of age, all 5 remaining puppies were negative for *Ehrlichia* spp., *Anaplasma* spp., and *B. burgdorferi* antibodies and *D. immitis* antigen by SNAP 4Dx Plus ELISA.

4 | DISCUSSION

Documentation of coinfection with *M. haemocanis* and *E. ewingii* in a pregnant dog just before parturition provided a unique opportunity to assess perinatal transmission of these pathogens. In the absence of any known vector exposure, infection with *M. haemocanis* was documented in 1 of 5 surviving puppies at 1 month of age, after which the puppy remained infected through 5 months of age. Although unlikely, it is possible that vertical transmission of *M. haemocanis* to the other puppies occurred and infection was below the level of PCR detection. It is reasonable to suspect vertical transmission of hemotropic *Mycoplasma* spp. to these puppies as this is an important mode of transmission for other red blood cell parasites including *B. canis* and *B. gibsoni* in dogs, and malaria in humans.[38,39] With only 1 of 6 puppies demonstrating *M. haemocanis* DNA, and with *M. haemocanis* not amplified from multiple tissues from the puppy that died shortly after birth, widespread transplacental transmission seems unlikely, but cannot be ruled out.[20,40] Although the 1 placenta sample that was available was PCR positive for *M. haemocanis*, infection in the puppy that it belonged to was not detected. Thus, other possible routes of perinatal transmission include transvaginal or transmammary transmission, direct transmission from blood-to-blood contact during parturition, or indirect transmission from exposure to infected saliva or feces within the first month of life. Although no fleas or ticks were seen on daily examinations, it also remains possible that vector transmission of *M. haemocanis* occurred in a single puppy.

Based on ELISA results, all 5 surviving puppies had maternal transfer of *Ehrlichia* spp. antibodies. These antibodies were present in 1-month-old puppies, but had waned by the time puppies reached 2 months of age. Although there is no *E. ewingii* IFA test, the SNAP 4Dx Plus *Ehrlichia* spp. test contains an *E. ewingii*-specific peptide.[41,42] Thus, the *Ehrlichia* spp. seroreactivity was presumably to *E. ewingii*, based on the positive ELISA, negative *E. canis* IFA, and positive *E. ewingii* PCR from the dam. There was no serological or PCR evidence supporting perinatal transmission of *E. ewingii* from the dam to the puppies.

While the dam was PCR positive for *B. clarridgeiae* at 3 months postpartum, at no point did she develop cross-reactive antibodies against *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, or *B. koehlerae*. Seemingly, this antibody specificity is consistent with experimental infection with *B. henselae* and *B. vinsonii* subsp. *berkhoffii*, in which dogs developed antibodies against the species and strain they were infected with, but did not develop cross-reactive antibodies to other species or strains.[43] It is well recognized that documentation of *Bartonella* spp. bacteremia by whole blood PCR is highly insensitive, because of the low number or lack of organisms (relapsing bacteremia).[44] When and how this dam became infected by *B. clarridgeiae* was not determined by our testing; however, flea exposure before hospitalization seems a likely source of *B. clarridgeiae* infection. Alternatively, exposure may have occurred while the dam was housed with cats at the veterinary clinic (although no direct contact was noted to occur with the resident cats, and no ectoparasites were seen on daily examinations).

The dam was *R. rickettsii* seroreactive both prepartum and at the 5-month time-point. Because there she was PCR negative and had no acute illness compatible with Rocky Mountain spotted fever, and because evidence supporting persistent infection with spotted fever group *Rickettsia* spp. in North America is lacking, we presume that the antibody response was associated with previous exposure to a low pathogenicity spotted fever group rickettsial organism. With a history of infestation with both fleas and *R. sanguineous*, and presumed previous exposure to *A. americanum* (the vector of *E. ewingii*), simultaneous or sequential infection with *Rickettsia felis* or *Rickettsia amblyommii* is a possibility. The positive test for *D. immitis* was suspected to be a very low-level infection, since the following month the test was negative with no intervening treatment, but 1 year later the test was positive again.

In conclusion, this study supports a potential role for vertical transmission in the maintenance of *M. haemocanis* in dogs as reservoir hosts. In this single case, there was no evidence that *E. ewingii* was transmitted transplacentally or during the perinatal period.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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