Use of enrofloxacin for treatment of large-form *Haemobartonella felis* in experimentally infected cats

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Objective—To compare treatment with enrofloxacin and doxycycline with no treatment in cats experimentally infected with *Haemobartonella felis*.

Design—Prospective case-control study.

Animals—16 cats.

Procedure—Cats were inoculated with large-form *H felis* from a chronically infected donor. Cats were assigned to 1 of 4 treatment groups: doxycycline (5 mg/kg [2.3 mg/lb], PO, q 12 h), low-dose enrofloxacin (5 mg/kg, PO, q 24 h), high-dose enrofloxacin (10 mg/kg [4.5 mg/lb], PO, q 24 h), and an untreated control group. Clinical signs, Hct, blood smears, and a polymerase chain reaction (PCR) assay were used to monitor progression of the infection.

Results—All cats were confirmed to be infected with *H felis* via blood smear evaluations and PCR assay results. Treatment had no effect on Hct during the intratreatment period, but Hct values were significantly greater in the low-dose enrofloxacin group, compared with the control group, during the posttreatment period. During the intratreatment period, *H felis* organism counts per 1,000 RBC in the doxycycline treatment and the high-dose enrofloxacin treatment groups decreased at a significantly faster rate than those in the control group. In the posttreatment period, organism counts in the doxycycline treatment group and the low- and high-dose enrofloxacin groups decreased at significantly faster rates than counts in the control group. There was no significant effect of treatment on the number of positive PCR assay results. Two cats treated with enrofloxacin and 1 cat treated with doxycycline completely cleared the *H felis* organism despite presumed immunosuppression caused by glucocorticoids.

Conclusions and Clinical Relevance—Results support the hypothesis that enrofloxacin has anti-*H felis* effects. (*J Am Vet Med Assoc* 2002;221:250–253)

Haemobartonella felis, a gram-negative epicellular rod, is the causative agent of feline infectious anemia, a hemolytic anemia of cats that can result in fever, lethargy, anorexia, splenomegaly, anemia, icterus, and death.[1] The organism was originally classified in the family Anaplasmataceae, order Rickettsiales. Recent sequencing of 16s rRNA, however, suggests that the organism is more closely related to Mycoplasmataceae.[2-4] It has been proposed that the 2 strains, previously known as *H felis* small form and *H felis* large-form, be reclassified in the genus Mycoplasma as *Candidatus* Mycoplasma haemominutum and *Candidatus* Mycoplasma haemofelis, respectively.[5,6]

Tetracyclines, and specifically doxycycline, have been used as standard treatments for *H felis* infection. Although the spectrum of action of tetracyclines includes *Mycoplasma* and *Ureaplasma* species, studies[7-9] have revealed that cats treated with tetracyclines recover clinically but are chronic carriers of *H felis*. Additionally, 8 of 29 experimentally infected cats had recurrent episodes of clinical signs and laboratory abnormalities, including parasitemia (6 cats) and anemia (8), after treatment with doxycycline.[10] Other anti-*Mycoplasma* antibiotics, including enrofloxacin and azithromycin, have been proposed for the treatment of haemobartonellosis.[11-12] However, azithromycin appeared ineffective in controlling clinical disease or clearing the organism at the dosing regime studied in 1 group of experimentally infected cats.[12]

The fluoroquinolones are used clinically to treat a variety of *Mycoplasma* spp infections.[13-15] The pharmacokinetics, volume of distribution, and clinical therapeutic regimens for enrofloxacin use in cats have been established.[16] To our knowledge, however, only anecdotal evidence exists for enrofloxacin as a treatment for haemobartonellosis.[11] The purpose of the study reported here was to compare treatment with enrofloxacin and doxycycline with no treatment in cats experimentally infected with large-form *H felis*, with respect to clinical signs, duration and severity of anemia, and clearance of the *H felis* organisms (evaluated cytologically and by use of **polymerase chain reaction [PCR]** assay).

Materials and Methods

The following protocol and animal treatment was reviewed and approved by the Colorado State University Animal Care and Use Committee in compliance with federal guidelines.

Cats—Eighteen specific pathogen-free cats (7 castrated males and 11 sexually intact females) between the ages of 1 and 5 years were used in the study. Two of the 18 cats served as donor cats for the *H felis* organism. The remaining 16 cats were randomly assigned to 1 of 4 treatment groups, for a total of 4 cats/group. Cats were acclimated to laboratory housing for at least 4 weeks prior to the start of the study. A physical

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examination, CBC, serum biochemical analyses, FeLV antigen test,[a] feline immunodeficiency virus antibody test,[b] and an *H felis* PCR assay[17,c] were performed to rule out other concurrent disease and previous infection by *H felis*. Cats were housed together for the duration of the study, and food (dry feline maintenance diet) and water were available ad libitum. All cats were adopted out to private homes at the end of the study.

Method of infection—Twenty-five days prior to the start of the study, the 2 donor cats were inoculated IV with 1 ml of heparinized blood from a chronically infected carrier of *H felis*. In addition, each donor was given methylprednisolone acetate (20 mg, IM) to maximize bacteremia. A CBC and *H felis* PCR assay were performed on **postinoculation (PI)** day 24 to confirm the presence of *H felis* in the donor cats. Both cats were confirmed as infected with *H felis* on the basis of cytologic evaluation and results of PCR assay, and 26 ml of blood was obtained in heparinized syringes from each cat and mixed aseptically. Each of the 16 study cats was then inoculated IV with 3 ml of the mixed donor blood.

Treatment groups—The 16 cats were allocated into 4 treatment groups: doxycycline (5 mg/kg [2.3 mg/lb], PO, q 12 h), low-dose enrofloxacin[d] (5 mg/kg, PO, q 24 h), high-dose enrofloxacin (10 mg/kg [4.5 mg/lb], PO, q 24 h), and no treatment (control group). Control group cats were to be treated with antibiotics only if deemed necessary as a life-saving measure. All cats receiving antibiotics were treated for a total of 14 days.

Protocol—The study duration was approximately 8 weeks (54 days). Blood samples were collected from cats on PI days 0, 7, 14, 21, 25, 28, 32, 35, 39, 42, and 54. At each sampling, 1.5 ml was placed in an EDTA tube and submitted to the clinical pathology laboratory for CBC and cytologic evaluation. The same clinical pathologist (CO), who was blinded to the treatment groups, reviewed all blood samples. An additional 1 ml of whole blood was placed in a separate EDTA tube and stored at 4 C for 5 to 7 days until *H felis* PCR analysis was performed. Daily scoring, which included body temperature, heart rate, respiration rate, mucous membranes, attitude, and appetite, was recorded by the same clinical scorer who was blinded to the treatment groups. In addition, cats were assessed daily by the same veterinarian (KLD) to ensure that cats did not need immediate medical attention; however, as this veterinarian was not blinded to the treatment groups, these assessments were not included in the daily score. Starting at PI day 11, daily PCV (via centrifugation) were performed for 1 week and then every other day for the remainder of the study. In an attempt to equate treatment responses to characteristic clinical abnormalities, treatment was started in individual cats on the first day their PCV was < 25% or a fever > 104.0 F (40.0 C) was noticed. These values were chosen as typical for those that would induce clinical illness that would be recognized by an owner and prompt them to seek veterinary care for the affected cat. If anemia or fever were not present in the treatment-group cats by day 28 of the study, treatment was initiated to assess the effect on clearance of *H felis* in cats that became infected but remained asymptomatic. Cats were evaluated for 8 weeks after inoculation, as previous studies[7,10] have reported that although antibiotics may result in clinical improvement and temporary clearance of the organism during antibiotic treatment, cats again have positive PCR assay results within days to weeks of discontinuation of the antibiotics. Cats that had positive PCR assay results by PI day 54 were eliminated from the study.

Immunosuppression—Cats that had negative PCR assay results by PI day 54 were administered glucocorticoids and evaluated for an additional 3 week. Cats with negative PCR assay results were given methylprednisolone acetate (20 mg/kg [9.1 mg/lb], IM) weekly until they had positive PCR assay results or for a total of 3 doses, whichever came first. Blood samples for cytologic assessment and PCR assay were obtained twice weekly during the immunosuppression phase of the experiment only on cats with negative PCR assay results. Cytologic assessment and PCR assay were also performed on these cats at 6 months after inoculation.

Clinical pathology—During the 4-week preinfection acclimation period, Wright-Giemsa stained blood smears (at least 1,000 to 2,000 RBC) were examined microscopically for the presence of *H felis* at 1,000× under oil immersion at 3 different times. All samples were considered negative. Postinfection parasitemia was determined by use of the same protocol and was recorded as the number of parasitized RBC/1,000 RBC. Red blood cells were considered parasitized if 1 or more basophilic coccoid bodies, ring forms (singly or in chains), or epicellular rods were seen. Cytologic evaluation was performed on PI days 0, 7, 14, 21, 25, 28, 32, 35, 39, 42, and 54.

Polymerase chain reaction assay—Polymerase chain reaction was performed on DNA extracted from blood in EDTA by use of a previously reported protocol.[17,c] The PCR assay was performed within 5 to 7 days of the sample date in batches of 16 (1 sample from each cat). Measures to prevent cross-contamination of DNA were maintained, and appropriate positive and negative controls were included in each PCR assay.

Statistical analyses—Intra- and posttreatment Hct values were analyzed by use of a mixed ANOVA model appropriate for a repeated-measures experiment, using commercially available software.[e] Time was included in the model as a continuous variable. The effect of treatment on the presence or absence of *H felis* by use of PCR assay during the intra- and posttreatment periods was evaluated by use of the Fisher exact test for binomial data. For assessment of *H felis* counts/1,000 RBC, data were transformed (log10 [count +1]) prior to analysis. Transformed data were analyzed as described for the Hct data. In a separate analysis, the number of days following infection that each cat developed a Hct < 30% were calculated and analyzed by use of the Wilcoxon 2-sample test (1-sided comparisons). This value was chosen for the data analysis because a Hct of < 30% at this institution's altitude is considered suspect. Values of $P < 0.05$ were considered significant.

Results

Clinical findings—All 16 cats developed at least 1 clinical sign (fever, icterus, lethargy, signs of depression, inappetence, or pale mucous membranes) consistent with haemobartonellosis. Prior to infection, all cats had a Hct > 30% (mean, 37.4%). All cats had a Hct of < 30% (considered suspect at this institution's altitude), but only 12 of the 16 cats had a Hct below the more stringent treatment cutoff criterion of 25%. Fever (> 102.5 F [39.2 C]) was documented in 9 of 16 cats, but no cats had fevers above the treatment cutoff point of 104.0 F (40.0 C). Six of the 9 cats had 2 reported days of fever, but consecutive days of fever were evident in only 1 cat. Episodes of fever did not correspond to other clinical signs, and the clinical scorer uniformly reported these cats as being stressed during the temperature-taking procedure. Lethargy and signs of depression were documented consistently in 1 control cat for a period of 8 days. Pale mucous membranes

were detected in 6 cats, and icterus was reported in 5 cats. Increased heart rate was reported in 1 cat in the control group. None of the cats required blood transfusions. Clinical signs were minimal after antibiotic treatment was initiated. None of the control group required antibiotic treatment, which would have been given as a life-saving measure, but 2 cats were administered balanced electrolyte solution subcutaneously for several consecutive days because of inappetence. Clinical data were not analyzed quantitatively because of the small numbers and short duration of abnormal results. However, untreated cats were abnormal (signs of depression, inappetence, lethargy) for a greater number of days than treated cats. Drug toxicosis was not detected clinically in any cat.

Anemia—The preinoculation Hct for the 16 cats was not significantly different among the 4 treatment groups (mean, 37.4%). Although the control group had a lower mean Hct over time, compared with the other groups, only the mean Hct in the low-dose enrofloxacin group was significantly ($P = 0.022$) different from that of the control group.

When the mean number of days of anemia, defined as Hct < 30%, was compared among treatment groups, the low-dose enrofloxacin treatment group (4.5 days; $P = 0.034$) and the high-dose enrofloxacin treatment group (6; $P = 0.033$), but not the doxycycline treatment group (10; $P = 0.25$), had significantly fewer days of anemia, compared with the control group (15.5). However, significant differences among the doxycycline and enrofloxacin treatment groups were not detected.

***Haemobartonella felis* organism counts/1,000 RBC**—All cats had cytologic evidence of *H felis* infection on at least some PI days (range, 2 to 10 days out of 11 sample days; mean, 5.25 days). During antibiotic treatment, organism counts in the doxycycline treatment group ($P = 0.03$) and the high-dose enrofloxacin treatment group ($P = 0.025$) decreased at a faster rate than counts in the control group. After antibiotics were discontinued, organism counts in the doxycycline treatment group ($P = 0.016$), the low-dose enrofloxacin treatment group ($P = 0.026$), and the high-dose enrofloxacin treatment group ($P = 0.05$) decreased at a faster rate than counts in the control group. Comparisons among the treatment groups (eg, enrofloxacin vs doxycycline), however, were not significantly different.

Polymerase chain reaction assay—All cats tested positive for *H felis* on PCR assay within 7 days after inoculation. Treatment had no significant effect on the incidence of *H felis* as detected by PCR assay during antibiotic treatment or after antibiotics were discontinued. However, 3 cats (2 in the high-dose enrofloxacin group and 1 in the doxycycline group) persistently tested negative (on PCR assay) after treatment. These cats still tested negative 6 months after the end of the study despite administration of glucocorticoids.

Discussion

All cats in this study were successfully infected with *H felis*. Clinical findings were similar to those previously described following experimental inoculation with large-form *H felis*.[1,8,10] However, the mild anemia (4 of the cats failed to develop Hct < 25%) and short duration of clinical abnormalities in several of the cats may have affected statistical assessment of some results. Variation in the severity of response among cats could be related to several factors. Organism attenuation can occur with serial passage through several cats, but this seems unlikely in this study since several of the cats developed severe anemia. While all cats received the same volume of inoculum, it is possible that the dose of viable organism varied among cats. However, under normal circumstances using a pooled inoculum sample, it is reasonable to assume that the organism is randomly distributed. Host factors can play a role in the severity of clinical signs. Although all cats in this study were young adults, they were not age-matched. Age-related immunity has been suspected to affect response to infection in a variety of other diseases.

Cats in both enrofloxacin treatment groups had significantly fewer days of anemia after inoculation than those in the control group. Additionally, all 3 treatment groups cleared *H felis* from infected cells more rapidly than untreated cats on the basis of cytologic assessment. Although not analyzed statistically, the control cats appeared sicker, and 2 of the 4 required fluid therapy, whereas none of the treatment cats required any medical intervention. These results suggest that administration of enrofloxacin or doxycycline is beneficial in the treatment of haemobartonellosis in cats. The failure to find a significant difference for mean Hct or duration of anemia between the doxycycline treatment group and the control group, for mean Hct between the enrofloxacin groups and the control group during antibiotic treatment, and for mean Hct or duration of anemia between doxycycline and enrofloxacin treatment groups may simply reflect the small sample size and variation of severity of anemia in individual cats. Thus, we believe that we are unable to make accurate statements as to which treatment protocol is optimal based on these data.

Three of the 16 cats persistently tested negative on PCR (at least 6 months after completion of the study) for *H felis* following treatment with enrofloxacin (2 cats in the high-dose group) or doxycycline (1 cat). To our knowledge, these are the first documented cases of sustained clearance of *H felis* in experimentally infected cats in response to antibiotic treatment.[7-10] Organism clearance was documented in the Foley study,[10] in which 3 cats tested negative during treatment with doxycycline (2.5 mg/kg [1.1 mg/lb], PO, q 12 h) for 21 days. However, the cats tested positive again within 14 days after treatment ended. Several explanations may account for the different outcomes between studies.

The degree of virulence of the large-form *H felis* that is used may affect response to treatment. Clinical findings were more severe in the Foley study,[10] suggesting increased virulence and potentially explaining failure to induce organism clearance. However, this seems unlikely because treatment with antibiotics also fails to clear the less virulent small form *H felis*, on the basis of findings on blood smears or PCR assay results.[10-12] It is

also possible that different strains within the large-form *H felis* species have different antibiotic susceptibilities, independent of strain virulence. However, to the authors' knowledge, such a strain-specific antibiotic resistance pattern has not been investigated for *H felis*. In this study, doxycycline was used at a higher concentration but for a shorter treatment duration than in the Foley study,[10] potentially explaining the sustained clearance observed in our doxycycline-treated cat. Because enrofloxacin has not been used in a controlled setting, efficacy comparisons cannot be made. It is possible that enrofloxacin is superior to doxycycline for induction of organism clearance. Sensitivities of the PCR assays used in different studies may affect assessment of clearance results; it is possible that the persistently negative PCR assay results described here may simply represent a lack of sensitivity rather than a true negative status. However, this is unlikely based on findings in sensitivity experiments[17] and the finding that our 3 cats persistently tested negative for at least 6 months after antibiotic treatment in the face of presumed immunosuppression. The dose of methylprednisolone acetate administered to these cats has been reported to inhibit lymphocyte blastogenesis in adult cats.[18] It is possible that although the organism was apparently cleared from blood, it may have been sequestered in tissues like the spleen.[19] Finally, host-related factors may have played a role in the apparent clearance of organism from these 3 cats.

Acute blindness has been reported following administration of enrofloxacin in cats; the reported incidence rate is 0.0017%.[f] This complication of treatment may factor into a decision whether to use this drug as a first line of treatment for *H felis* infection in cats or as a rescue treatment in those cats failing to respond to tetracyclines. However, toxicity potential must be considered in all therapeutic decisions. For example, oral administration of doxycycline tablets has been associated with esophageal stricture formation in some cats.[20]

Results of this study support the hypothesis that enrofloxacin has anti-*H felis* effects. A study in which a larger number of cats per treatment group are used is indicated to further investigate the significance of these preliminary findings.

[a]FeLV antigen test, Snap Combo, IDEXX Laboratories, Portland, Me.
[b]FIV antibody test, Snap Combo, IDEXX Laboratories, Portland, Me.
[c]*Haemobartonella felis* (large form) PCR, Heska Diagnostic Laboratory, Fort Collins, Colo.
[d]Baytril, Bayer Animal Health, Merriam, Kan.
[e]SAS MIXED procedure, version 8, SAS Institute Inc, Cary, NC.
[f]Olsen J, Bayer Veterinary Services, Fort Collins, Colo: Personal communication, 2001.

References

1. VanSteenhouse JL, Taboada J, Millard JR. Feline hemobartonellosis. *Compend Contin Educ Pract Vet* 1993;15:535–545.
2. Rikihisa Y, Kawahara M, Wen B, et al. Western immunoblot analysis of *Haemobartonella muris* and comparison of the 16s rRNA gene sequences of *H. muris*, *H. felis*, and *Eperythrozoon suis*. *J Clin Microbiol* 1997;35:823–829.
3. Neimark H, Kocan KM. The cell wall-less rickettsia *Eperythrozoon wenyonii* is a *Mycoplasma*. *FEMS Microbiol Lett* 1997; 156:287–291.
4. Johansson KE, Tully JG, Boelske G, et al. *Mycoplasma cavipharyngis* and *Mycoplasma fasitidiosum*, the closest relatives to *Eperythrozoon* spp. and *Haemobartonella* spp. *FEMS Microbiol Lett* 1999;174:321–326.
5. Foley JE, Pedersen NC. '*Candidatus* Mycoplasma haemominutum', a low-virulence epierythrocytic parasite of cats. *Int J Sys Evol Microbiol* 2001;51:815–817.
6. Neimark H, Johansson KE, Rikihisa Y, et al. Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of '*Candidatus* Mycoplasma haemofelis', '*Candidatus* Mycoplasma haemomuris', '*Candidatus* Mycoplasma haemosuis' and '*Candidatus* Mycoplasma wenyonii'. *Int J Sys Evol Microbiol* 2001;51:891–899.
7. Berent LM, Messick JB, Cooper SK. Detection of *Haemobartonella felis* in cats with experimentally induced acute and chronic infections, using a polymerase chain reaction assay. *Am J Vet Res* 1998;59:1215–1220.
8. Harvey JW, Gaskin JM. Experimental feline haemobartonellosis. *J Am Anim Hosp Assoc* 1977;13:28–38.
9. Harvey JW, Gaskin JM. Feline haemobartonellosis: Attempts to induce relapses of clinical disease in chronically infected cats. *J Am Anim Hosp Assoc* 1978;14:453–456.
10. Foley JE, Harrus S, Poland A, et al. Molecular, clinical, and pathologic comparison of two distinct strains of *Haemobartonella felis* in domestic cats. *Am J Vet Res* 1998;59:1581–1588.
11. Winter RB. Using quinolones to treat hemobartonellosis (lett). *Vet Med Small Anim Clin* 1993;88:306–307.
12. Westfall DS, Jensen WA, Reagan WJ, et al. Inoculation of two genotypes of *Hemobartonella felis* genotypes (California and Ohio variants) to induce infection in cats and the response to treatment with azithromycin. *Am J Vet Res* 2001;62:687–691.
13. Friis NF, Szancer J. Sensitivity of certain porcine and bovine mycoplasmas to antimicrobial agents in a liquid medium test compared to a disc assay. *Acta Vet Scand* 1994;35:389–394.
14. Bahri LE, Blouin A. Fluoroquinolones: a new family of antimicrobials. *Compend Contin Educ Pract Vet* 1991;13:1429–1433.
15. Bemis DA. *Bordetella* and *Mycoplasma* respiratory infections in dogs and cats. *Vet Clin North Am* 1992;22:1173–1186.
16. Richez P, Monlouis JD, Dellac B, et al. Validation of a therapeutic regimen for enrofloxacin in cats on the basis of pharmacokinetic data. *J Vet Pharmacol Ther* 1997;20(suppl 1):152–153.
17. Jensen WA, Lappin MR, Kamkar S, et al. Prevalence of *Haemobartonella felis* infection in cats. *Am J Vet Res* 2000;62:604–608.
18. Lappin MR, Dawe DL, Lindl PA, et al. The effect of glucocorticoid administration on oocyst shedding, serology, and cell-mediated immune responses of cats with acute or chronic toxoplasmosis. *J Am Anim Hosp Assoc* 1992;27:625–632.
19. Maede Y. Sequestration and phagocytosis of *Haemobartonella felis* in the spleen. *Am J Vet Res* 1979;40:691–695.
20. Melendez LD, Twedt DC, Wright M. Suspected doxycycline induced esophagitis with esophageal stricture formation in three cats. *Feline Pract* 2000;28:10–12.