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Efficacy of Enrofloxacin or Doxycycline for Treatment of *Bartonella henselae* or *Bartonella clarridgeiae* Infection in Cats

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Enrofloxacin and doxycycline are antimicrobial agents used to treat bacterial diseases of cats. In vitro susceptibility data indicate that either drug should be effective against *Bartonella* species. In vivo efficacies of these drugs for eradication of chronic *Bartonella henselae* or *Bartonella clarridgeiae* infections were examined in 18 experimentally infected cats and 25 naturally exposed cats treated with enrofloxacin (22.7 mg given orally [PO] every 12 h [q12h] [14 days, $n = 10$; 28 days, $n = 13$]) or with doxycycline (25 mg PO q12h [14 days, $n = 9$; 28 days, $n = 8$]) or not treated ($n = 3$). Plasma drug concentrations were determined in experimental cats by high-performance liquid chromatography. Only 23 of 43 cats enrolled ultimately met inclusion criteria. Bacteremia was eliminated for 12 to 25 weeks posttreatment in four of seven cats receiving 14 days of enrofloxacin, five of seven cats receiving 28 days of enrofloxacin, one of six cats receiving 14 days of doxycycline, and one of two cats receiving 28 days of doxycycline. Defining a negative result by blood culture as treatment success may be erroneous; these results may reflect the insensitivity of blood culture or the relapsing nature of *Bartonella* bacteremia. Our results suggest that MICs obtained with axenic media do not predict antimicrobial activity against intracellular *Bartonella*, that a long treatment course is required to eliminate infection, and that duration of therapy correlates with pretreatment bacterial load. Given current concern about the development of antimicrobial resistance, we would reserve recommendation for treatment to cats owned by an immunocompromised individual or as an alternative to euthanasia of a pet.

Bartonella species are gram-negative, facultative organisms that cause multisystemic infectious diseases in humans. *Bartonella* infections are being recognized with increased frequency because of identification of *Bartonella*-specific diseases (bacillary angiomatosis, peliosis hepatis, peliosis splenitis, endocarditis, etc.) in immunocompromised and immunocompetent people and the subsequent development of more precise diagnostic tests. The spectrum of human disease caused by *Bartonella* species has expanded considerably in recent years. In particular, the potential role of *Bartonella henselae* in the development of AIDS dementia is receiving attention from the medical community. Cats are a zoonotic reservoir of *B. henselae* and *Bartonella clarridgeiae. B. henselae* is the predominant cause of classical cat scratch disease (CSD) but has also been associated with bacillary angiomatosis, granulomatous disorders, and endocarditis (2, 13, 36). A high prevalence of asymptomatic feline infection with *B. henselae* has been reported by several investigators (9, 11, 16, 17). A more recently identified species, *B. clarridgeiae*, was implicated as the cause of CSD in one person, but the potential pathogenic role of this organism in people is currently unknown (21, 23). Our laboratory identified 7 of 70 feline *Bartonella* isolates as *B. clarridgeiae*, suggesting that among *Bartonella*-infected cats in North Carolina, *B. clarridgeiae* may account for approximately 10% of isolates. Both species appear to cause persistent infections in cats that are not accompanied by obvious clinical abnormalities. In view of the high prevalence of infection and the chronic persistence of *Bartonella* bacteremia in cats, elimination of infection by antimicrobial therapy could substantially decrease the potential for bacterial transmission between cats or to people. This issue is of particular relevance for immunocompromised cat owners. A definitive therapeutic strategy for elimination of *Bartonella* infection in cats has not been reported.

In vitro, *Bartonella* species are susceptible to several antimicrobial agents including erythromycin, chloramphenicol, rifampin, gentamicin, trimethoprim-sulfamethoxazole, doxycycline, and ciprofloxacin (27, 28, 30). However, there is poor correlation between in vitro susceptibility and in vivo responsiveness to many antibiotics. Due to the intracellular association of these organisms with feline erythrocytes and human pericytes and endothelial cells, in vitro MIC results obtained by using blood agar do not appear to correlate with in vivo antimicrobial efficacy (20, 24, 26). Numerous individual case reports indicate successful or unsuccessful treatment of *B. henselae* infections in people with the same aforementioned antibiotics (6, 15, 25, 29). In many instances, an extended duration of therapy was necessary to obtain complete clinical recovery. However, spontaneous disease resolution could have accounted for the recovery. Little is known about the preferred cellular localization of *B. henselae* or *B. clarridgeiae* following infection of cats or people.

In this study, the efficacies of doxycycline and enrofloxacin were investigated for several reasons: doxycycline is well tolerated by cats, can be administered orally, and has been used successfully in treating some people infected with *Bartonella* (25, 29). Doxycycline is also effective against rickettsial pathogens (e.g., *Haemobartonella felis*) and has activity against other gram-negative organisms. Enrofloxacin, a veterinary fluoroquinolone, is partially, but rapidly metabolized to ciprofloxacin in most animals (16). In vitro, ciprofloxacin is active against *Bartonella* species, and it has eradicated *Bartonella* infections in people (15, 25). *B. henselae* has been observed in human macrophages, neutrophils, pericytes, and endothelial cells (24). Fluoroquinolones achieve high drug concentrations in leukocytes, macrophages, and several other tissues and organs frequently colonized by *Bartonella* organisms, including the skin,

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spleen, liver, lymph nodes, heart, and bone. High concentrations of enrofloxacin and ciprofloxacin are attained in saliva and urine shortly after oral administration (3, 4). *Bartonella* species are associated with erythrocytes; therefore, cats with retrovirus-induced stomatitis or idiopathic hematuria might excrete organisms in these body fluids. In fact, *Bartonella* DNA has been detected in cat saliva (11), and viable organisms can be cultured from urine samples from cats experimentally inoculated with bartonella in vitro (22).

Although we have described the intraerythrocytic presence of *B. henselae* in bacteremic cats, precise cellular predilections of *B. henselae* and *B. clarridgeiae* in cats are currently unknown (18). To our knowledge, data related to the ability of doxycycline, enrofloxacin, or ciprofloxacin to penetrate feline erythrocytes are not available. However, doxycycline is soluble in lipids and should cross cell membranes readily, while fluoroquinolones are known to passively diffuse into bacteria and mammalian cells (4, 31).

MATERIALS AND METHODS

Therapeutic groups. Eighteen specific-pathogen-free cats (Liberty Research, Waverly, N.Y.) were challenged with blood samples from two donor cats naturally infected with *B. henselae* or *B. clarridgeiae* (20). Both donor cats were believed to be the source of *Bartonella*-induced CSD in their owners. Experimentally induced cats were monitored for 276 days as a component of a previously reported experimental infection study prior to initiation of this treatment protocol (20). Cats were housed in an American Association for Accreditation of Laboratory Animal Care-approved insect vector-free facility. In addition, 25 pet cats, many previously identified as being naturally infected with *Bartonella* species as a component of a previous study, were treated in a placebo-blinded crossover treatment trial (17). Mean weights of experimentally and naturally infected cats were 2.8 and 4.0 kg, respectively. Cats were randomly assigned an antibiotic (enrofloxacin or doxycycline) and treatment duration (14 or 28 days). Within the group of experimentally infected cats, eight received enrofloxacin (14 days, $n = 4$; 28 days, $n = 4$), and two received no treatment. Among the naturally infected cats, 15 received enrofloxacin (14 days, $n = 6$; 28 days, $n = 9$), 9 received doxycycline (14 days, $n = 5$; 28 days, $n = 4$), and 1 received a placebo (dextrose). Neither experimentally nor naturally infected cats were infected with the feline immunodeficiency virus or feline leukemia virus based upon the results of the CITE Combo enzyme-linked immunosorbent assay test kit (IDEXX, Portland, Maine).

Antimicrobial agents. Enrofloxacin at a dosage of 22.7 mg per cat every 12 h (q12h) (Baytril; Bayer Corp. Animal Health, Shawnee Mission, Kans.) or doxycycline hyclate at a dosage of 25 mg per cat q12h (Danbury Pharmacal, Danbury, Conn.) were administered orally. Experimentally infected cats received a mean dosage of 7.59 or 9.4 mg of enrofloxacin or doxycycline, respectively, per kg of body weight q12h. Naturally infected cats received a mean dosage of 5.42 or 7 mg of enrofloxacin or doxycycline, respectively, per kg q12h. Cat owners administered treatment at home and returned the cats periodically for follow-up examination and blood cultures.

***Bartonella* strains.** The type strain of *B. henselae* (Houston-1; ATCC 49882) was obtained from the American Type Culture Collection, Rockville, Md. The *B. clarridgeiae* type strain (ATCC 51734) was provided by R. S. Weyant, Centers for Disease Control and Prevention, and the *Bartonella vinsonii* subsp. *berkhoffii* type strain (ATCC 51672) was cultured by us from a dog with endocarditis (8, 19). *B. clarridgeiae* 94-F40 (ATCC 700095) and all other feline strains of *B. henselae* and *B. clarridgeiae* were isolated by our laboratory (17, 21). *Bartonella* strains derived from cats enrolled in this study and used for susceptibility testing were cultured from blood obtained immediately prior to initiation of antibiotic therapy and after treatment failure.

Antimicrobial susceptibility testing. *Bartonella* type strains and feline isolates were assayed in triplicate for in vitro susceptibility to doxycycline, enrofloxacin, and ciprofloxacin by the agar dilution method (37). Solutions of doxycycline, enrofloxacin, and ciprofloxacin were prepared at a stock concentration of 640 μg/ml, and serial twofold dilutions made in sterile water to an endpoint of 0.30 μg/ml. Two milliliters of each working dilution was added to 18 ml of molten Trypticase soy agar (TSA) containing 5% rabbit blood. After gentle inversion, the mixture was poured into sterile petri dishes (100 by 15 mm) and allowed to solidify at room temperature. This resulted in an MIC testing range of 0.03 to 64.0 μg/ml. Plates containing drug-free rabbit blood agar were used as growth controls.

Analysis of plasma drug concentrations. Plasma samples were obtained from experimentally infected cats pretreatment and on day 6 (2, 5, and 7 h posttreatment), day 14 (2, 6, and 8 h posttreatment) for those animals receiving 14 days of therapy, and day 28 (2, 6, and 8 h posttreatment) for those animals receiving 28 days of therapy. Doxycycline, enrofloxacin, and the active metabolite for enrofloxacin, ciprofloxacin, were analyzed in plasma by reverse-phase high performance liquid chromatography (HPLC). For all analyses, the apparatus consisted of a Waters Model 600 Pump (Millipore Corp., Milford, Mass.). Hewlett-Packard Series 1050 Autosampler (Hewlett-Packard, Palo Alto, Calif.), UV detector (Hewlett-Packard), and computer for data collection and analysis (Hewlett-Packard HPLC^{2D} ChemStation running in Windows 3.1 on a Hewlett-Packard Vectra 486/33N computer). The column was a Zorbax SB-C8 (4.6 mm by 15 cm) (MAC-MOD Analytical Inc., Chadds Ford, Pa.), with a Zorbax RX-C18 guard column (4 mm by 1.25 cm) (MAC-MOD Analytical Inc.).

Doxycycline was eluted with a mobile phase consisting of 76% distilled water, 14% acetonitrile, and 10% methanol at a flow rate of 1.0 ml/min. To each liter of mobile phase, 6.84 g of oxalic acid was added (0.1 M). The mobile phase was filtered and degassed prior to use. Doxycycline was detected at a wavelength of 350 nm. Enrofloxacin, ciprofloxacin, and the internal standard norfloxacin were eluted with a mobile phase consisting of 81% distilled water, 19% acetonitrile, and 0.02% trifluoroacetic acid (TFA) at a flow rate of 1.0 ml/min. The mobile phase was filtered and sparged with helium prior to use. These fluoroquinolones were detected at a wavelength of 279 nm. The column temperature in all cases was 40°C.

Calibration curves were prepared in the following manner. A 1-mg/ml doxycycline stock solution was prepared by dissolving a pure reference standard of doxycycline in distilled water and kept refrigerated in a tightly sealed vial. The stock solution was diluted with distilled water to produce a range of spiking solutions from 1,000 to 2.5 μg/ml. Ten microliters of the spiking solution was added to 90 μl of untreated plasma to produce 10 calibration plasma samples ranging from 100.0 to 0.25 μg/ml. Stock solutions (1 mg/ml) of enrofloxacin, ciprofloxacin, and the internal standard norfloxacin were prepared by dissolving pure reference standards in 0.1% TFA and kept refrigerated in a tightly sealed vial. Spiking solutions of enrofloxacin and ciprofloxacin were prepared by diluting stock solutions with a mixture of methanol–0.1% TFA (15:85) to make concentrations ranging from 1,000 to 5 μg/ml. The internal standard spiking solution was prepared by diluting the norfloxacin stock solution in a mixture of methanol–0.1% TFA (15:85) to a concentration of 10 μg/ml. Untreated plasma samples were used as blanks.

Prior to analysis for doxycycline concentration, plasma samples from the study, as well as the calibration plasma samples, were prepared by mixing 100 μl of the sample with an equivalent amount of a plasma protein releasing agent that consisted of 20% acetonitrile, 2% phosphoric acid, and 78% distilled water. This admixture was vortexed for 10 s, pipetted into a 10,000-molecular-weight cutoff microcentrifuge tube (Ultrafree-MC, 10,000 nominal molecular weight limit [NMWL] centrifugal filter unit; Millipore Corp.) and centrifuged for 30 min at 14,000 × *g*. The clear filtrate was transferred to an HPLC injection vial. In order to determine enrofloxacin and ciprofloxacin concentrations, 300 μl of each plasma sample from the study, as well as each prepared calibration plasma sample, was pipetted into a clean glass tube, followed by the addition of 20 μl of the internal standard solution and 5 μl of acetonitrile. The tube was centrifuged at 600 × *g* for 10 min at room temperature. The supernatant was transferred to a clean glass tube, and the contents were evaporated under a flow of nitrogen (20 lb/in^2) at 40°C for 30 min. The residue in each tube was reconstituted with 200 μl of a mixture of methanol–0.1% TFA (15:85) and transferred to an HPLC injection vial. The injection volume was 50 μl for doxycycline and fluoroquinolone analysis.

Calculations and statistical analysis. A new calibration curve was prepared for each day's samples. Approximately 24 samples were analyzed each day. Only linear calibration curves with an r^2 value of at least 0.99 were accepted. The calibration standards were back-calculated to within 15% of the true value. The retention time for elution of doxycycline was 6 to 6.5 min. The limit of quantification (LOQ) for doxycycline, defined as 10× baseline noise, was approximately 0.5 μg/ml. Unknown concentrations were calculated from the response (in milliabsorbance units [mAU]) using the calibration curve. The retention times for norfloxacin, ciprofloxacin, and enrofloxacin were 3.8 to 3.9, 4.3 to 4.4 and 5.6 to 5.8 min, respectively. The LOQ for both enrofloxacin and ciprofloxacin was approximately 0.05 μg/ml. Unknown concentrations were calculated by taking the ratio of either enrofloxacin or ciprofloxacin to the internal standard and plotting the ratio against standards.

Plasma drug concentrations among sampling periods were compared to assess the effect of treatment duration on plasma drug concentration by a two-way analysis of variance (ANOVA) on the SAS program with a level of significance of $P = 0.05$ (SAS Institute, Cary, N.C.). Elimination half-lives were estimated from the slopes (k) of the plasma drug concentration-versus-time curves. The half-life was calculated to be $0.693/k$. Elimination rates among treatment periods were compared by a two-factor ANOVA on the SAS computer program.

Therapeutic result. Blood was drawn by jugular venipuncture from all cats at predetermined time intervals to assess the effectiveness of antibiotic therapy. Samples were obtained from experimental cats immediately prior to the first treatment and at 2- to 3-week intervals for 12 to 25 weeks posttherapy. Pet cats were tested at the time of enrollment in the study (treatment day 1) and at 3, 5, 9, 12, and 24 weeks after initiation of the treatment protocol. Blood was aliquoted for lysis centrifugation blood culture (1.5-ml Pediatric Isolator tubes; Wampole Laboratories, Cranbury, N.J.), anticoagulation treatment with EDTA (2.0 ml), and serum collection (2.5 ml). Blood cultures were incubated on TSA with 5% rabbit blood at 35°C in 5% CO_2 for 60 days. EDTA-treated blood samples were used to determine complete blood counts and were also analyzed

TABLE 1. Antibiotic susceptibilities of *Bartonella* type strains and feline isolates obtained by the agar dilution method

Bartonella species (strain or cat)[a]	Treatment regimen[b]	Posttreatment culture result[c]	MIC (μg/ml) (posttreatment MIC)[d]		
			Doxycycline	Enrofloxacin	Ciprofloxacin
B. henselae (ATCC 49882)	NA	NA	0.125	0.5	0.5
B. clarridgeiae (ATCC 51734)	NA	NA	0.125	0.125	0.5
B. clarridgeiae (ATCC 700095)	NA	NA	0.125	0.125	0.5
B. vinsonii subsp. *berkhoffii* (ATCC 51672)	NA	NA	0.125	0.5	0.5
B. henselae (cat 2, exp.)	2wD	Neg	0.125	ND	ND
B. henselae (cat 3, exp.)	2wD	Pos	0.125 (0.125)	ND	ND
B. henselae (cat 1, nat.)	2wD	Pos	0.125 (0.125)	ND	ND
B. henselae (cat 2, nat.)	2wD	Pos	0.125 (0.125)	ND	ND
B. henselae (cat 3, nat.)	2wD	Pos	0.125 (0.125)	ND	ND
B. clarridgeiae (cat 4, exp.)	2wD	Pos	0.125 (0.125)	ND	ND
B. henselae (cat 4, nat.)	4wD	Pos	0.125 (0.125)	ND	ND
B. henselae (cat 5, nat.)	4wD	Neg	0.125	ND	ND
B. henselae (cat 5, exp.)	2wE	Pos	ND	0.5 (0.25)	0.5 (0.25)
B. henselae (cat 6, exp.)	2wE	Neg	ND	1.0	1.0
B. henselae (cat 6, nat.)	2wE	Pos	ND	0.5 (0.25)	0.5 (0.25)
B. henselae (cat 7, nat.)	2wE	Neg	ND	0.5	0.5
B. henselae (cat 8, nat.)	2wE	Pos	ND	0.5 (0.5)	0.5 (0.5)
B. henselae (cat 9, nat.)	2wE	Neg	ND	0.5	0.5
B. henselae (cat 10, nat.)	2wE	Neg	ND	0.5	0.5
B. henselae (cat 12, nat.)	4wE	Neg	ND	0.5	0.5
B. henselae (cat 13, nat.)	4wE	Pos	ND	0.5 (0.5)	0.5 (0.5)
B. henselae (cat 14, nat.)	4wE	Neg	ND	0.5	0.5
B. henselae (cat 11, nat.)	4wE	Neg	ND	0.5	0.5
B. clarridgeiae (cat 15, nat.)	4wE	Neg	ND	0.125	0.5
B. clarridgeiae (cat 7, exp.)	4wE	Neg	ND	0.125	0.5

[a] The ATCC designations of type strains are given in parentheses. *Bartonella* was isolated from naturally (nat.) and experimentally (exp.) infected cats.
[b] NA, not applicable; 2wD, 2 weeks of doxycycline; 4wD, 4 weeks of doxycycline; 2wE, 2 weeks of enrofloxacin; 4wE, 4 weeks of enrofloxacin.
[c] NA, not applicable; Neg, negative; Pos, positive.
[d] MICs shown are the final MIC results on day 10. MICs for strains isolate from treatment failures (next positive culture) are shown in parentheses. ND, not done.

by PCR for the presence of *Bartonella* DNA. Eubacterial primers were used to amplify bacterial ribosomal DNA from blood samples and from blood culture isolates as previously described (21, 38). Resultant amplicons were examined by restriction fragment length polymorphism analysis to confirm the identity of the bacterium as a *Bartonella* species and to identify instances of reinfection with a heterologous subspecies or species (21). Either a positive blood culture result or amplification of *Bartonella* DNA by PCR was recorded as an indication of bacteremia. In both naturally and experimentally infected cats, therapeutic failure was defined as bacteremia detected within the 12- to 25-week posttreatment period. Serum was evaluated by an indirect fluorescent-antibody assay (IFA) for *Bartonella*-specific immunoglobulin G by a protocol described elsewhere (17).

RESULTS

Antimicrobial susceptibility testing. Due to slow growth of several isolates on drug-free blood agar plates, final MIC results were not obtainable on day 5 but were recorded on day 10 postinoculation. All *Bartonella* type strains and feline isolates obtained immediately prior to administration of antimicrobials were susceptible to doxycycline, enrofloxacin, and ciprofloxacin (Table 1). Doxycycline, at 0.125 μg/ml, inhibited growth of all *Bartonella* strains tested. Enrofloxacin and ciprofloxacin effectively suppressed growth of all *B. clarridgeiae* strains at 0.125 and 0.50 μg/ml, respectively. The fluoroquinolones inhibited growth of all but one strain of *B. henselae* at 0.50 μg/ml. The MIC of enrofloxacin or ciprofloxacin for this strain was 1.0 μg/ml. *Bartonella* strains cultured from treatment failures did not demonstrate increased resistance to the antimicrobials in the test panel.

Plasma drug concentrations. HPLC analysis of plasma samples from treated, experimental cats confirmed that concentrations of drug in plasma at day 6 and day 14 or 28 of treatment attained levels considered to be therapeutic (Table 2). Doxycycline concentrations were well above the LOQ of 0.5 μg/ml in each plasma sample assayed. A portion of the enrofloxacin was metabolized to ciprofloxacin. Enrofloxacin and ciprofloxacin concentrations were above the LOQ of 0.05 μg/ml in each plasma sample assayed. Mean ciprofloxacin concentrations were 19% (± standard error of the mean, 1.6%) of the corresponding plasma enrofloxacin concentration at each time point. Only concentrations at the 2-h time points were identical for each of the three sampling periods (days 6, 14, and 28). Elimination half-lives estimated from the slope of the plasma drug concentration curves ranged from 5.6 to 8.2 h for enrofloxacin and 7.4 to 9.5 h for doxycycline. Among sampling periods, there were no significant differences in the elimination rates of doxycycline, enrofloxacin, and ciprofloxacin in plasma when measured on days 6, 14, and 28.

Clinical outcome. During the course of the study, indications of illness were not observed in the naturally or experimentally infected cats. Aural and rectal temperature measurements,

TABLE 2. Plasma drug concentrations after oral administration of doxycycline or enrofloxacin

Time	Mean drug concn (μg/ml) (SE)		
	Doxycycline	Enrofloxacin	Ciprofloxacin
Day 6, 2 h	3.82 (0.52)	4.72 (0.78)	0.62 (0.08)
Day 6, 5 h	2.94 (0.38)	3.23 (0.57)	0.57 (0.07)
Day 6, 7 h	2.36 (0.27)	2.56 (0.45)	0.53 (0.06)
Day 14, 2 h	3.60 (0.29)	3.23 (0.78)	0.48 (0.02)
Day 14, 6 h	2.94 (0.16)	2.12 (0.12)	0.50 (0.04)
Day 14, 8 h	2.14 (0.58)	1.61 (0.19)	0.44 (0.04)
Day 28, 2 h	3.59 (0.74)	3.66 (0.72)	0.50 (0.02)
Day 28, 6 h	3.05 (0.72)	2.82 (0.28)	0.55 (0.03)
Day 28, 8 h	2.13 (0.55)	2.08 (0.38)	0.46 (0.04)

TABLE 3. *Bartonella* blood culture and serology results following antibiotic treatment of experimentally infected cats that satisfied criteria

Cat[a]	*Bartonella* species[b]	Treatment regimen[c]	Dose (mg/kg)	Test[d]	Test result[e] at the following no. of wks posttreatment:									
					0	2	4	6	9	12	16	19	22	25
1	Bh	None	0.0	Culture	+	+	+	+	+	+	+	−	−	+
				PCR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
				IFA titer	<16	32	<16	<16	<16	<16	<16	<16	<16	<16
2	Bh	2wD	6.9	Culture	+	−	−	−	−	−				
				PCR	−	−	−	−	−	−				
				IFA titer	512	512	1,024	1,024	512	512				
3	Bh	2wD	12.8	Culture	+	−	+	−	−	−	−	+	+	+
				PCR	+	ND	ND	ND	−	−	−	ND	ND	ND
				IFA titer	32	64	64	32	64	<16	<16	<16	128	64
4	Bc	2wD	10.2	Culture	+	−	−	+	+	+	+	+	+	+
				PCR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
				IFA titer	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
5	Bh	2wE	8.6	Culture	+	+	+	+	−	−	−	+	+	+
				PCR	−	ND	ND	ND	−	−	−	ND	ND	ND
				IFA titer		256	512	1,024	256	512	128	256	1,024	256
6	Bh	2wE	6.7	Culture	+	−	−	−	−	−				
				PCR	−	ND	ND	−	−	−				
				IFA titer	512	512	512	256	64	128				
7	Bc	4wE	11.4	Culture	+	−	−	−	−	−				
				PCR	−	ND	ND	−	−	−				
				IFA titer	<16	<16	32	<16	<16	<16				

[a] Cats 2, 6, and 7 were monitored for 12 weeks and then randomly assigned to groups in another study.
[b] Bh, *B. henselae*; Bc, *B. clarridgeiae*.
[c] 2wD, 2 weeks of doxycycline; 2wE, 2 weeks of enrofloxacin; 4wE, 4 weeks of enrofloxacin.
[d] Reciprocal IFA titers reflect seroreactivity to the *Bartonella* species with which the cat was infected.
[e] +, positive; −, negative; ND, not done.

pulse, and respiration rate were within established reference ranges. Complete blood count results derived from experimentally infected cats failed to identify anemia (packed cell volume, <30%) or leukocytosis in any cats, but three cats were eosinophilic. Only two cats appeared to experience mild adverse drug reactions. One experimentally infected cat and one naturally infected cat had intermittent vomiting during the administration of enrofloxacin. However, both of these cats became culture negative after treatment.

Efficacy, or lack thereof, was assessed by blood culture and PCR analysis of blood for *Bartonella* DNA. Although 43 cats initially enrolled in this study were treated and monitored, the ultimate inclusion of data was dependent upon the detection of *Bartonella* bacteremia (culture or PCR) in blood samples obtained immediately before antimicrobials were administered and our subsequent ability to obtain several follow-up examinations for at least 12 weeks. For various reasons, only 7 of 18 experimentally infected cats and 16 of 25 naturally infected cats satisfied these criteria. Treatment success, defined as the failure to detect bacteremia by culture or DNA by PCR following treatment, occurred in 9 of 14 enrofloxacin recipients and 2 of 8 doxycycline recipients (Tables 3 and 4). An experimentally infected cat that received no treatment remained intermittently bacteremic for an additional 25 weeks, while the other untreated cat was abacteremic at the start of the study. Some cats maintained high, stable immunoglobulin G titers during the entire observation period but gave negative results for *Bartonella* bacteremia by blood culture and PCR. In other cats, *Bartonella*-specific antibodies to both *B. henselae* and *B. clarridgeiae* antigens were not detected, despite periodic isolation of *B. henselae* or *B. clarridgeiae.*

DISCUSSION

The emergence of *B. henselae* and *B. clarridgeiae* as causes of CSD and other disseminated disease processes in humans has resulted in efforts to selectively reduce the reservoir of bartonella in the cat population. The purpose of this study was to evaluate the efficacies of enrofloxacin and doxycycline in the treatment of chronic *B. henselae* and *B. clarridgeiae* infections in cats. At its inception, the design seemed very straightforward. Previously documented bacteremic cats would be treated with antimicrobials and subsequently monitored by blood culture and PCR analysis to establish the clearance of *Bartonella* organisms. Cats with negative blood cultures and negative PCR results were interpreted as having a successful therapeutic outcome. However, shortly before beginning the study, we documented relapsing bacteremia in cats experimentally infected with either *B. henselae* or *B. clarridgeiae* and continuously housed in an insect vector-free isolation facility (19). Evaluation of treatment or vaccination strategies against *B. henselae* or *B. clarridgeiae* will be difficult due to unpredictable fluctuations in detectable bacteremia associated with the natural course of infection. On two separate occasions, we transmitted *B. henselae* from blood culture-negative cats by simultaneously inoculating blood into specific-pathogen-free cats (20). In addition, although DNA was extracted from EDTA-treated blood samples in triplicate, the sensitivity of PCR anal-

TABLE 4. *Bartonella* blood culture and serology results following antibiotic treatment of naturally infected cats that satisfied criteria

Cat	*Bartonella* species[a]	Treatment regimen[b]	Dose (mg/kg)	Test[c]	Test result[d] at the following no. of wks posttreatment:					
					0	3	5	9	12	24
1	Bh	2wD	8.9	Culture	+	+	−	+	+	+
				PCR	ND	ND	ND	ND	ND	ND
				IFA titer	128	128	128	128	128	256
2	Bh	2wD	10.4	Culture	+	−	+	+	+	ND
				PCR	ND	ND	ND	ND	ND	ND
				IFA titer	64	32	64	64	64	ND
3	Bh	2wD	12.5	Culture	+	−	+	+	+	+
				PCR	+	ND	ND	ND	ND	ND
				IFA titer	512	256	256	256	256	512
4	Bh	4wD	10.4	Culture	+	−	−	−	−	+
				PCR	ND	ND	ND	−	−	−
				IFA titer	64	64	64	32	64	64
5	Bh	4wD	4.0	Culture	+	−	−	−	−	ND
				PCR	ND	−	−	−	−	ND
				IFA titer	32	<16	<16	32	<16	ND
6	Bh	2wE	5.7	Culture	+	+	ND	−	+	ND
				PCR	ND	ND	ND	ND	ND	ND
				IFA titer	256	256	ND	32	64	ND
7	Bh	2wE	6.3	Culture	+	−	ND	−	−	ND
				PCR	−	ND	ND	−	−	ND
				IFA titer	1,024	1,024	ND	1,024	512	ND
8	Bh	2wE	6.3	Culture	+	+	ND	+	+	ND
				PCR	ND	ND	ND	ND	ND	ND
				IFA titer	32	64	ND	128	128	ND
9	Bh	2wE	6.0	Culture	−	−	−	−	−	ND
				PCR	+	ND	ND	−	−	ND
				IFA titer	32	64	32	64	32	ND
10	Bh	2wE	6.0	Culture	−	−	−	−	−	ND
				PCR	+	ND	ND	−	−	
				IFA titer	32	32	16	64	32	ND
11	Bh	4wE	4.4	Culture	−	−	−	−	−	−
				PCR	+	ND	ND	−	−	−
				IFA titer	512	512	512	512	512	512
12	Bh	4wE	6.0	Culture	+	+	−	−	−	ND
				PCR	+	ND	ND	−	−	ND
				IFA titer	512	512	256	512	256	ND
13	Bh	4wE	5.7	Culture	+	+	−	+	+	ND
				PCR	−	ND	ND	−	−	ND
				IFA titer	1,024	1,024	512	512	512	ND
14	Bh	4wE	6.7	Culture	+	−	−	−	−	ND
				PCR	ND	ND	ND	ND	ND	ND
				IFA titer	1,024	256	256	256	256	ND
15	Bc	4wE	6.3	Culture	+	−	−	−	−	−
				PCR	ND	ND	−	−	−	−
				IFA titer	<16	<16	<16	<16	<16	<16
16	Bc	4wE	3.5	Culture	+	−	−	+	+	ND
				PCR	+	ND	ND	ND	ND	ND
				IFA titer	128	128	64	64	128	ND

[a] Bh, *B. henselae*; Bc, *B. clarridgeiae*.
[b] 2wD, 2 weeks of doxycycline; 4wD, 4 weeks of doxycycline; 2wE, 2 weeks of enrofloxacin; 4wE, 4 weeks of enrofloxacin.
[c] Reciprocal IFA titers reflect seroreactivity to the *Bartonella* species with which the cat was infected.
[d] +, positive; −, negative; ND, not done.

ysis of blood containing low numbers of bacteria was inherently poor because only 100 μl of blood was used in the assay. These factors illustrate the ease with which false-negative culture or PCR results could create confusion in the interpretation of experimental treatment trials in cats. Since there are no reliable clinical signs or clinicopathologic indicators of *Bartonella* infection in cats, physical examination and laboratory results are not helpful in assessing antimicrobial efficacy (20). Similarly, the correlation of immunoglobulin concentration to *Bartonella* bacteremia in cats is highly variable, thereby limiting the utility of serology for establishing therapeutic efficacy. Given the difficulties in establishing a diagnosis of bartonella infection in cats, several cats previously identified as bacteremic with *Bartonella* on multiple occasions prior to the initiation of treatment were ultimately eliminated from the data analysis when blood obtained immediately prior to the initiation of therapy failed to yield evidence of bacteremia.

In naturally exposed pet cats, two additional confounding factors may have contributed to the therapeutic outcome. First, owner compliance must be considered. Other than a statement of intent to comply with the treatment regimen, which was signed at the inception of the study, there was no pharmacologic verification that the owner was administering the prescribed medication. Second, with regard to the interpretation of positive follow-up blood cultures, the possibility of reinfection could not be eliminated. Naturally infected cats were continuously exposed to other known infected cats and to fleas, both of which are potential sources of *Bartonella* reinfection (10, 22). No method of analysis was available for determining reexposure to homologous *Bartonella* strains. In addition, due to the close relatedness of *Bartonella* species, the molecular analysis of the isolates obtained in this study lacked the sensitivity to distinguish heterologous strains within the same species. However, PCR-restriction fragment length polymorphism patterns of isolates obtained following treatment were identical to pretreatment results. Therefore, reinfection with a heterologous *Bartonella* species or subspecies was considered unlikely.

Treatment was ineffective in some experimentally infected cats despite therapeutic plasma drug concentrations that were reached within 2 h of oral administration and sustained for at least 8 h. Table 2 shows that plasma drug concentrations were maintained above the enrofloxacin and doxycycline MICs during each sampling period. In addition, since ciprofloxacin is expected to have an additive effect to enrofloxacin, these concentrations added approximately 20% to the antimicrobial activity of enrofloxacin at each sampling point.

One purpose of this study was to examine whether *Bartonella* infection or duration of treatment affected disposition of doxycycline or enrofloxacin in these cats. Since the first sampling period for drug analysis was not conducted until day 6, plasma drug concentrations were at steady state. As expected, once concentrations achieved steady state, they did not vary among sampling periods when measured at days 6, 14, and 28. In these cats, the peak enrofloxacin concentrations were 4.7 (± 0.78) to 3.23 (± 0.08) μg/ml at 2 h. Because the enrofloxacin had accumulated to steady-state levels, concentrations were higher in these cats than after administration of a single dose. In another report, the peak concentration of enrofloxacin after administration of a single oral dose of 5 mg/kg to cats was 1.9 (± 0.58) μg/ml at 2 h, but concentrations of ciprofloxacin were not measured (35). The peak doxycycline concentrations in these cats ranged from 3.82 (± 0.52) to 3.59 (± 0.74) μg/ml at 2 h. In a previous report, average concentrations at steady state after 5-mg/kg doses were predicted to be 6.37 μg/ml, but that was after intravenous drug administration (34).

Half-lives were not significantly different for either enrofloxacin or doxycycline among the three sampling periods. The half-lives for doxycycline and enrofloxacin, averaged for all sampling days, were estimated to be from 7.4 to 9.5 and 5.6 to 8.2 h, respectively. These estimates, which must be taken as approximate because they were measured from only three plasma drug concentrations, were longer than what has been reported previously. Doxycycline has been reported to have a half-life of 4.56 (± 0.68) h in healthy cats (34). The half-life of enrofloxacin in cats was previously reported to be 3.6 (± 0.48) h (35), and the manufacturer indicates a half-life greater than 4 h.

Based upon our results, as well as those of recent studies, MICs obtained by using axenic medium are not indicative of the therapeutic efficacy of antimicrobials against intracellular organisms such as *Bartonella* (28, 32). There are many problems related to documentation of the therapeutic elimination of intracellular organisms (14). For bacteriostatic drugs such as tetracyclines, the pathogen must be exposed to the drug during the entire dose interval. Since we have little information available regarding the cellular tropism of *B. henselae* and *B. clarridgeiae* in cats, the specific intracellular location(s) of these bacteria are likewise unknown. Alternatively, cells containing bartonella organisms may have impaired antimicrobial uptake or may store drugs in intracellular compartments that do not communicate with the bacteria.

Three other groups have examined the efficacy of antimicrobial therapy of *B. henselae* infection in cats, and although several factors make comparison of the data difficult, certain observations can be made relative to the collective findings. Greene et al. (12) and Regnery et al. (33) investigated antibiotic treatments during acute infection (28 days or less) in cats inoculated with laboratory-cultivated *B. henselae*. Dramatic reductions in colony counts were observed by Greene and coworkers immediately following the administration of doxycycline, amoxicillin, or clavulanate-potentiated amoxicillin, with all cats ultimately becoming blood culture negative. The cats were monitored for 4 weeks after treatment before eradication of infection was declared. Regnery et al. observed a more rapid reduction in colony counts from cats receiving tetracycline or erythromycin compared to counts from untreated controls, but a reduction in colony count was not observed in cats receiving enrofloxacin or amoxicillin. In their study, treated, as well as control, cats ultimately became culture negative and remained abacteremic during a 4-month observation period. Bergmans et al. (5) found that 5 weeks of combination therapy with doxycycline and erythromycin eliminated *Bartonella* infection in four of six experimentally infected cats, during a posttreatment observation period of 10 weeks. Although antagonism was not noted, whether combination therapy offered an advantage over monotherapy with either of these agents was not clearly established.

Although successful elimination of *B. henselae* infection has been reported in the studies described in the preceding paragraph, important differences should be noted regarding the design of our study. A potential important difference in study design relates to the source of inoculum, which may have contributed to differences in inoculum virulence. Greene et al. (12) and Regnery et al. (33) used laboratory-cultivated bartonella rather than inoculation of infected blood to experimentally infect cats prior to treatment. The short duration of bacteremia in cats receiving cultivated inoculum does not correlate well with the prolonged, and at times relapsing, bacteremia observed in naturally exposed cats, experimental cats receiving infected cat blood as inoculum, or cats infected through flea contact. Enhanced virulence of bacteria following

passage through an appropriate host or vector is not an uncommon phenomenon, particularly for intracellular pathogens. In contrast to other studies in which treatment was initiated during acute infection, the cats in this study were chronically bacteremic with *B. henselae* or *B. clarridgeiae*. Therefore, the relevance of our data may be more applicable to natural infections. Since infection with bartonella does not generally induce obvious clinical abnormalities in cats, veterinarians will more likely be treating chronic, previously unrecognized infections. Another concern regarding the establishment of antimicrobial efficacy in previous studies is the duration of posttreatment abacteremia used to designate cats as successfully cleared of *Bartonella* infection. In a controlled experimental setting, more than 4 months has elapsed between detectable periods of bacteremia by lysis centrifugation blood cultures in untreated cats that were infected via blood inoculum (22). Our results would have been much different if we had limited our posttreatment observation period of 12 or 25 weeks to 4 or 10 weeks. Therefore, based upon the results of this study, future studies investigating *Bartonella* antimicrobial efficacy should consider the type of inoculum used to establish infection, the duration of infection prior to initiation of treatment, and difficulties associated with confirming posttreatment elimination of the infection. As a result of these difficulties, a limitation of this and other studies was the small numbers of cats in the respective treatment groups.

Human data suggest that higher than normal doses in conjunction with a longer period of administration may be indicated for treatment of *Bartonella* infections. Because of the available formulation, the dosage of enrofloxacin used in this study was chosen for convenient administration. Although exceeding the manufacturer's recommendations, the dosages were within the safe range (1, 7). The dose of enrofloxacin used in our study was several times greater than the dose used by Regnery and coworkers (33). This may explain the relative efficacy of enrofloxacin in our study. Enrofloxacin produces a postantibiotic effect and is considered to be bactericidal, in contrast to doxycycline, which is considered bacteriostatic (31). Although only a limited number of cats was used, our results suggest that the level of pretreatment bacteremia also affects therapeutic outcome with enrofloxacin. Typically, enrofloxacin has not shown any significant inoculum effects over standard testing of antibacterial concentrations in vitro (3). An important finding from our data was that cats, like other animal species, convert enrofloxacin to ciprofloxacin. High concentrations of both enrofloxacin and ciprofloxacin in plasma were documented following oral administration of enrofloxacin to experimentally infected cats. Ciprofloxacin is an active metabolite and probably exerted an additive effect because ciprofloxacin concentrations were approximately 20% of the corresponding enrofloxacin concentrations at each sampling point. Although the optimal antimicrobial agent, dosage, and duration of treatment remain uncertain, enrofloxacin, administered at high doses and for a long duration (4 to 6 weeks) may hold promise for the treatment of *B. henselae* and *B. clarridgeiae* infections of cats. However, given current concern about the development of antimicrobial resistance, we would reserve recommendation for treatment to cats owned by an immunocompromised individual or as an alternative to euthanasia of a pet.

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