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Persistence of *Borrelia burgdorferi* in Experimentally Infected Dogs after Antibiotic Treatment

REINHARD K. STRAUBINGER,[1]* BRIAN A. SUMMERS,[2] YUNG-FU CHANG,[3] AND MAX J. G. APPEL[1]

James A. Baker Institute for Animal Health,[1] Department of Pathology,[2] and Diagnostic Laboratory,[3] College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

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In specific-pathogen-free dogs experimentally infected with *Borrelia burgdorferi* by tick exposure, treatment with high doses of amoxicillin or doxycycline for 30 days diminished but failed to eliminate persistent infection. Although joint disease was prevented or cured in five of five amoxicillin- and five of six doxycycline-treated dogs, skin punch biopsies and multiple tissues from necropsy samples remained PCR positive and *B. burgdorferi* was isolated from one amoxicillin- and two doxycycline-treated dogs following antibiotic treatment. In contrast, *B. burgdorferi* was isolated from six of six untreated infected control dogs and joint lesions were found in four of these six dogs. Serum antibody levels to *B. burgdorferi* in all dogs declined after antibiotic treatment. Negative antibody levels were reached in four of six doxycycline- and four of six amoxicillin-treated dogs. However, in dogs that were kept in isolation for 6 months after antibiotic treatment was discontinued, antibody levels began to rise again, presumably in response to proliferation of the surviving pool of spirochetes. Antibody levels in untreated infected control dogs remained high.

Lyme disease, or Lyme borreliosis, in humans and domestic animals is caused by the tick-transmitted spirochete *Borrelia burgdorferi*. The clinical signs in humans were reviewed by Steere (43), and those in dogs were reviewed by Levy et al. (17). There appears to be a general agreement that *B. burgdorferi* persists in humans and animals for months or years, and perhaps for life (3, 5, 29, 41), despite a strong humoral immune response. One possible explanation for the persistence is the failure of the host to produce borreliacidal OspA antibodies (34, 35, 44), which are induced by vaccination but rarely following natural infection. Another possible explanation is the invasion of poorly vascularized connective tissues by spirochetes or even an intracellular localization (10, 13, 16, 18). Elimination of borreliae by antibiotic treatment would be highly desirable. Although *B. burgdorferi* is susceptible to antibiotics in vitro (4, 14), there are contradictory reports as to the in vivo efficacy of antibiotics. While some authors claim that elimination of *B. burgdorferi* occurs after antibiotic treatment of human patients (20, 25), others are less certain (30, 37, 45). The antibiotics most commonly used for the treatment of acute Lyme disease in humans are orally administered doxycycline and amoxicillin (26, 32, 40). Intravenous delivery of antibiotics is reserved for chronic and persistent disease (8, 46).

A variety of animal models have been used to study the efficacy of antibiotic treatments. As in humans, contradictory results have been obtained in mice (19, 23), hamsters (14, 15), and gerbils (31). We have developed a canine model of Lyme borreliosis that in its clinical signs, pathology, persistence of spirochetes, and immune response closely resembles many aspects of human Lyme borreliosis (3). We have used the canine model to evaluate the effectiveness of doxycycline and amoxicillin in eliminating both bacterial persistence and joint lesions and to evaluate the effects of antibiotic therapy on the immune response to *B. burgdorferi.*

* Corresponding author. Mailing address: James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853. Phone: (607) 256-5637. Fax: (607) 256-5608. E-mail: rks4@cornell.edu.

MATERIALS AND METHODS

Dogs. Specific-pathogen-free (SPF) beagles from the Baker Institute colony were used. Dogs were kept in P2 isolation units in conformance with the Animal Welfare Act and New York State Department of Health regulations. The experiments were initiated in 6-week-old male and female pups. The dogs were observed daily for clinical signs. The body temperature and body weight of each pup were tested daily and weekly, respectively.

Infection of dogs. The dogs were infected by placing 15 adult female and 7 male ticks onto the clipped side of each dog for a 7-day period as reported previously (3). Ticks (*Ixodes scapularis*) were collected by flagging in a forested area in North Salem, Westchester County, N.Y., in April and November. The infectivity of the ticks was confirmed by culturing 20 individual ground ticks from each collection in BSK-II medium for 6 weeks as reported previously (3). Of 20 ticks collected in the spring, 10 were culture positive, and of 20 ticks collected in the fall, 12 were culture positive. Two SPF beagles served as uninfected control dogs.

Antibiotic treatment. Approximately 2 months after tick exposure, by which time infection was documented by *B. burgdorferi* isolation and positive PCR results from skin biopsies taken at the sites of the tick bites, antibiotic treatment was initiated and maintained for a 30-day period. One dog (A95-2/5) remained uninfected. Six dogs received 50 mg (approximately 10 mg/kg of body weight) of doxycycline twice a day orally; four dogs received 100 mg (approximately 20 mg/kg) of amoxicillin three times a day orally; two dogs received the same amount of amoxicillin twice a day orally (A94-10/3 and 10/7); and six infected dogs remained untreated. After the antibiotic treatments were completed, the dogs were kept for an additional 2 (A94-10 series) to 6 (A95-2 series) months before euthanasia was performed. Additional skin punch biopsy samples were taken during this time (see Tables 1 and 2).

Isolation of *B. burgdorferi*. For isolation of *B. burgdorferi*, skin punch biopsy samples (4-mm diameter) were collected from dogs at approximately monthly intervals before and after antibiotic treatment and at 2-week intervals for some dogs (see Tables 1 and 2). In addition, 23 different tissues were collected from each dog at necropsy, with frequent changes of instruments to avoid cross-contamination. These tissues included synovial membranes from six joints (shoulder, elbow, and stifle), muscle and fascia from the front and hind limbs, cervical, axillary, and popliteal lymph nodes, pericardium, peritoneum, and meninges. Skin biopsy samples were ground in 0.2 ml of BSK-II + KR medium with a pellet pestle and placed into 6.5 ml of medium as reported previously (3). Postmortem tissues were suspended in medium in a tissue homogenizer (stomacher; Teckmar, Cincinnati, Ohio). Three milliliters of the suspension was placed into 27 ml of prewarmed BSK-II + KR medium as reported previously (3). The medium was incubated at 34°C for 5 weeks and examined at 1, 3, and 5 weeks by dark-field microscopy for the presence of live spirochetes.

PCR. (i) DNA extraction. To avoid cross contamination with DNA products from previous PCRs, extraction, amplification, and detection of DNA were carried out in separated rooms with different sets of equipment. Total DNA from skin punch biopsies, from postmortem tissues, and from cultured bacteria were extracted according to a standard protocol (36) with minor modifications. Briefly, tissues or pelleted bacteria were digested in a solution containing 100 μl of

TABLE 1. Culture demonstration of persistence of *B. burgdorferi* in experimentally infected and antibiotic-treated dogs

Dog no.	Treatment	Skin punch biopsy sample[a]								Postmortem tissue sample[a]
		Before initiation of treatment[b]		After initiation of treatment[c]						
		1	2	3	4	5	6	7	8	
A94-10/2	Doxycycline	+	+	−	−	−	−	−	−	+
A94-10/4	Doxycycline	+	+	−	−	−	−	−	−	−
A95-2/0	Doxycycline	−	+	−	−	−				−
A95-2/1	Doxycycline	+	+	−	−	−	−	−	+	−
A95-2/4	Doxycycline	+	+	−	−	−	−	−	−	−
A95-2/8	Doxycycline	+	+	−	−	−	−	−	−	−
A94-10/3	Amoxicillin	+	+	−	−	−	−	−	−	−
A94-10/7	Amoxicillin	+	+	−	−	−	−	−	−	+
A95-2/2	Amoxicillin	+	+	−	−	−	−	−	−	−
A95-2/3	Amoxicillin	+	+	−	−	−	−	−	−	−
A95-2/5[d]	Amoxicillin	−	−	−	−	−	−	−	−	−
A95-2/9	Amoxicillin	+	+	−	−	−	−	−	−	−
A94-10/1	None	+	ND	+	ND	+	+	ND	+	+
A94-10/5	None	+	ND	+	ND	+	+	ND	+	+
A94-10/6	None	+	ND	+	ND	+	+	ND	+	+
A94-10/8	None	−	ND	−	ND	−	+	ND	+	+
A95-2/6	None	+	+	+	ND	+	−	ND	+	+
A95-2/10	None	+	+	+	ND	+				+

[a] +, presence of *B. burgdorferi* DNA; −, absence of *B. burgdorferi* DNA; ND, not done.
[b] Samples 1 and 2 were taken 16 and 1 day, respectively, before initiation of treatment in group A94-10 and 33 and 5 days, respectively, before initiation of treatment in group A95-2.
[c] Samples 3 to 8 were taken 12, 40, 54, 68, 82, and 97 days, respectively, after initiation of treatment in group A94-10 and 9, 23, 51, 134, 162, and 191 days, respectively, after initiation of treatment in group A95-2.
[d] Tick-exposed, uninfected dog.

proteinase K (10 mg/ml; Gibco BRL, Grand Island, N.Y.), 150 μl of 5% sodium dodecyl sulfate (Amersham Life Science, Cleveland, Ohio), and 75 μl of β-mercaptoethanol (Sigma, St. Louis, Mo.) in a 1.5-ml microcentrifuge tube for 3 h. DNA was extracted with Tris-saturated phenol (pH = 8.0; Amersham Life Science), chloroform, and isoamyl alcohol (Fisher Scientific, Pittsburgh, Pa.). Purified DNA was precipitated with 7.5 M ammonium acetate and 70% ethanol, dried, and dissolved in water. Concentration and purity of extracted DNA were verified at two wavelengths, λ_1 = 260 nm and λ_2 = 280 nm.

(ii) PCR. Previously published primer sets for the chromosomal 23S rRNA gene and for the plasmidial OspA gene were chosen for the detection of *B. burgdorferi* (JS1, 5′-AGAAGTGCTGGAGTCGA-3′, and JS2, 5′-TAGTGCTC TACCTCTATTAA-3′ [38]; and SL1, 5′-AATAGGTCTAATAATAGCCTTAA TAGC-3′, and SL2, 5′-CTAGTGTTTTGCCATCTTC TTTGAAAA-3′ [9]). Reactions were carried out in 50-μl volumes containing 1× buffer II, 1.5 mM $MgCl_2$, 200 μM (each) deoxynucleoside triphosphates, 1.25 U of *Taq* polymerase (Perkin Elmer, Branchburg, N.J.), 50 pmol of each primer, and 2 μl of DNA solution. The 23S rRNA gene amplification included heating to 94°C for 3 min, amplification during 45 cycles at 94°C for 45 s and at 50°C for 105 s, and extension at 72°C for 2 min. The OspA gene amplification included heating to 94°C for 2 min; amplification during 45 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; and extension at 72°C for 2 min (GeneAmp PCR System 9600; Perkin Elmer). DNA fragments were separated on 1.5% agarose gels, stained with ethidium bromide, and visualized over a UV light source.

Serology. Serum samples were collected from dogs at 2-week intervals beginning at the time of infection. Sera were tested for *B. burgdorferi* antibody levels by a computerized kinetic enzyme-linked immunosorbent assay (KELA) as reported previously (3). In order to avoid fluctuations between tests, all sera from each group of dogs were tested together.

Histopathology. Dogs were euthanized approximately 2 (A94-10 series) or 6 (A95-2 series) months after the antibiotic treatments had been finalized. Tissues were removed from euthanized dogs, fixed in 10% buffered formalin, and processed by standard methods. Tissues included joint capsules from the shoulder, elbow, carpus, stifle, and tarsus and the cervical, axillary, and popliteal lymph nodes.

Testing for antibiotic levels in dogs. On day 2 of the antibiotic treatment and again in weekly intervals until the end of treatment, blood samples were drawn into EDTA-coated blood collection tubes from dogs at 2, 4, 6, and 8 h after treatment from all antibiotic-treated dogs and again at 10 and 12 h after treatment from doxycycline-treated dogs. Plasma was collected. Plasma antibiotic concentrations were determined by an agar gel diffusion bioassay as reported previously (6). *Bacillus cereus* was used to test for doxycycline, and *Bacillus stearothermophilus* was used to test for amoxicillin (Difco Laboratories, Detroit, Mich.).

RESULTS

Clinical signs. Except for a few slight elevations in some dogs, the dogs' body temperatures remained normal throughout the experiment and weight gains were in the physiological range. One of the infected but untreated control dogs became lame and was euthanized for necropsy on day 120 postinfection (A95-2/10). One of the doxycycline-treated dogs (A95-2/0) showed signs of lameness and was euthanized 210 days after infection (109 days after the antibiotic treatment was completed).

Isolation of *B. burgdorferi*. Two months after tick exposure and before the antibiotic treatment of the dogs was initiated, *B. burgdorferi* was isolated from skin punch biopsy samples of all dogs with the exception of one amoxicillin-treated dog (A95-2/5) that remained uninfected throughout the experiment and one infected and untreated control dog (A94-10/8) that had positive skin biopsies at a later date (Table 1).

Within 2 weeks of initiation of the antibiotic treatment, skin punch biopsy samples from all treated dogs became culture negative while biopsy samples from untreated infected control dogs remained culture positive (Table 1). Skin punch biopsy samples from all treated dogs remained culture negative for the remaining 2 (A94-10 series) or 6 (A95-2 series) months until euthanasia with the exception of that from dog A95-2/1, which had a positive biopsy 6 months after doxycycline treatment.

Attempts to isolate *B. burgdorferi* from tissues collected postmortem 2 (A94-10 series) or 6 (A95-2 series) months after the antibiotic treatment had been completed were made. Results are presented in Table 1. *B. burgdorferi* was isolated from multiple tissues of six of six untreated infected control dogs but from only axillary lymph nodes (near the site of tick exposure) in one doxycycline (A94-10/2)- and one amoxicillin (A94-10/7)-treated dog (Table 1).

TABLE 2. PCR demonstration of persistence of *B. burgdorferi* in experimentally infected and antibiotic-treated dogs

Dog no.	Treatment	Skin punch biopsy sample[a]									Postmortem tissue sample[a]
		Before initiation of treatment[b]		After initiation of treatment[c]							
		1	2	3	4	5	6	7	8	9	
A94-10/2	Doxycycline	+	+	+	+	+	+	+	+		ND
A94-10/4	Doxycycline	+	+	+	+	−	+	+	−	+	ND
A95-2/0	Doxycycline	+	+	+	−	+	−				+
A95-2/1	Doxycycline	+	+	+	+	+	+	+	+	+	+
A95-2/4	Doxycycline	−	+	−	−	−	+	−	−	−	+
A95-2/8	Doxycycline	+	+	−	−	−	−	+	−	−	−
A94-10/3	Amoxicillin	+	+	+	+	−	−	+	+		ND
A94-10/7	Amoxicillin	+	+	+	−	+	+	−	+	+	ND
A95-2/2	Amoxicillin	+	−	−	−	+	+	−	+	−	−
A95-2/3	Amoxicillin	+	+	−	−	+	+	+	−	−	+
A95-2/5[d]	Amoxicillin	−	−	−	−	−	−	−	−	−	−
A95-2/9	Amoxicillin	+	+	+	−	+	+	−	−	+	+
A94-10/1	None	+	ND	+	ND	+	ND	+	ND	+	ND
A94-10/5	None	+	ND	+	ND	+	ND	−	ND	+	ND
A94-10/6	None	+	ND	+	ND	+	ND	+	ND	+	ND
A94-10/8	None	+	ND	+	ND	+	ND	+	ND	+	ND
A95-2/6	None	−	+	+	ND	−	ND	−	ND	+	+
A95-2/10	None	+	+	+	ND	+					+

[a] +, presence of *B. burgdorferi* DNA; −, absence of *B. burgdorferi* DNA; ND, not done.
[b] Samples 1 and 2 were taken 16 and 1 day, respectively, before initiation of treatment in group A94-10 and 33 and 5 days, respectively, before initiation of treatment in group A95-2.
[c] Samples 3 to 9 were taken 26, 40, 54, 68, 82, 97, and 117 days, respectively, after initiation of treatment in group A94-10 and 9, 23, 51, 107, 134, 162, and 191 days, respectively, after initiation of treatment in group A95-2.
[d] Tick-exposed, uninfected dog.

PCR. Skin punch biopsy and postmortem tissues were tested for borrelia DNA with specific primer pairs targeting the chromosomal 23S rRNA gene or the OspA gene on plasmids. The results are presented in Table 2 and Fig. 1. PCR was positive for skin punch biopsy samples from all dogs with the exception of dog A95-2/5 before antibiotic treatment was initiated. Within 2 weeks of initiation of the antibiotic treatment, skin punch biopsy samples from two of six doxycycline- and two of five amoxicillin-treated dogs became PCR negative while biopsy samples from untreated infected control dogs remained PCR positive. However, between 51 and 134 days after treatment was completed, skin punch biopsy samples from all infected and antibiotic-treated dogs showed positive PCRs again (Table 2; Fig. 1). In addition, with the exception of dog A95-2/5, which remained uninfected throughout the experiment, multiple tissues from three of four doxycycline-treated, two of three amoxicillin-treated, and two of two untreated infected dogs had positive PCRs (Table 2). Tissues from two uninfected SPF dogs, as well as from dog A95-2/5, that served as negative controls were PCR negative.

Joint histopathology. No significant joint lesions were seen in any of the amoxicillin-treated dogs by 2 (A94-10 series) or 6 months (A95-2 series) after antibiotic treatment was completed (4 and 8 months, respectively, after infection). The joints of five of six doxycycline-treated dogs were inconspicuous as well. However, one doxycycline-treated dog (A95-2/1) had mild joint lesions by 8 months after infection. Deep in the joint capsule of the right tarsus, light infiltrates of neutrophils, eosinophils, and monocytes were seen. A small leukocytic focus was found in the joint capsule of the right shoulder. Joint lesions were seen in four of six untreated infected control dogs. Mild to severe mono- or polyarthritis with plasma cells, lymphocytes, and a few neutrophils was seen in dogs A94-10/1, A94-10/5, A95-2/6, and A95-2/10. In untreated dogs, synovitis involved more joints and infiltrates were more severe than those for the single doxycycline-treated dog with joint lesions. Lymph node changes (e.g., cortical hyperplasia and sinus histiocytosis) were similar in all infected treated and untreated dogs.

The joints of two uninfected SPF control dogs were free of lesions.

Immune response. Positive KELA levels (above 100 U) appeared between 6 and 8 weeks after infection in all infected dogs with the exception of one amoxicillin-treated dog (A95-2/5) that remained uninfected. Thereafter, the titers in untreated infected control dogs increased and remained high until the dogs were euthanized 4 to 8 months after infection (Fig. 2C).

The antibody levels in antibiotic-treated dogs varied. KELA units declined in all antibiotic-treated dogs by 4 weeks after treatment was initiated and reached negative levels in four of six doxycycline- and four of five amoxicillin-treated dogs (one dog, A95-2/5, remained uninfected) (Fig. 2A and B). In dogs that were kept for 6 months after 30 days of antibiotic treatment, antibody levels began to rise again. Three of three doxycycline-treated dogs and two of three amoxicillin-treated dogs responded with increasing titers at that time (Fig. 2A and B).

Antibiotic levels in plasma. Peak concentrations in plasma were reached at 2 h after treatment (Fig. 3). MICs of doxycycline were still present in four of four dogs by 10 h after treatment and in two of four dogs by 12 h after treatment, when the antibiotic was administered again (Fig. 3A). MICs of amoxicillin were present at 4 h after treatment but not at 6 and

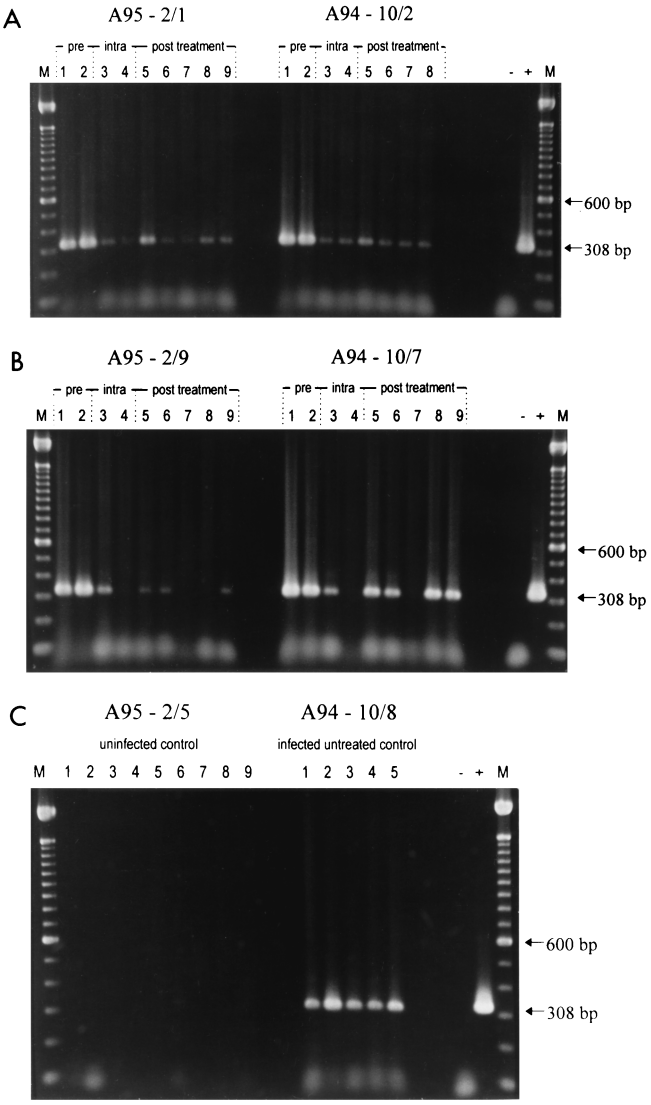

FIG. 1. Detection of *B. burgdorferi* in skin punch biopsy samples by PCR using a specific primer for the OspA gene. Lanes for all panels: M, molecular weight markers (the DNA fragment at 308 bp is specific for *B. burgdorferi* OspA); +, positive control using DNA from *B. burgdorferi* cultures; −, negative control using water. Doxycycline (A)- and amoxicillin (B)-treated dogs. Results are shown for dogs A95-2/1 and A95-2/9 33 and 5 days before initiation of treatment (lanes 1 and 2, respectively), and 9, 23, 51, 107, 134, 162, and 191 days after initiation of treatment (lanes 3 to 9, respectively). Results are also shown for dogs A94-10/2 and A94-10/7 16 and 1 days before initiation of treatment (lanes 1 and 2, respectively), and 26, 40, 54, 68, 82, 97, and 117 days after initiation of treatment (lanes 3 to 9, respectively). (C) For dog A95-2/5 (uninfected control dog), skin biopsy samples were tested over a 224-day period (lanes 1 to 9). For dog A94-10/8 (untreated infected control dog) skin biopsy samples were tested 34, 76, 104, 132, and 167 days after infection (lanes 1 to 5).

8 h after treatment, when treatment was repeated (Fig. 3B). Differences in plasma antibiotic levels were minimal when plasma antibiotic levels from different time intervals of treatment were compared.

DISCUSSION

An important question regarding Lyme disease remains: is conventional antibiotic treatment 2 to 4 weeks in duration sufficient to eliminate disease and infection? Disseminated Lyme disease may be associated with acute and/or long-term morbidity (22, 39), in which case the efficacy of the antibiotic treatment becomes very important. Some authors claim elimination of *B. burgdorferi* and disease occurs after antibiotic treatment of human patients (20, 25) while evidence is mounting that spirochetes can persist despite antibiotic therapy (12,

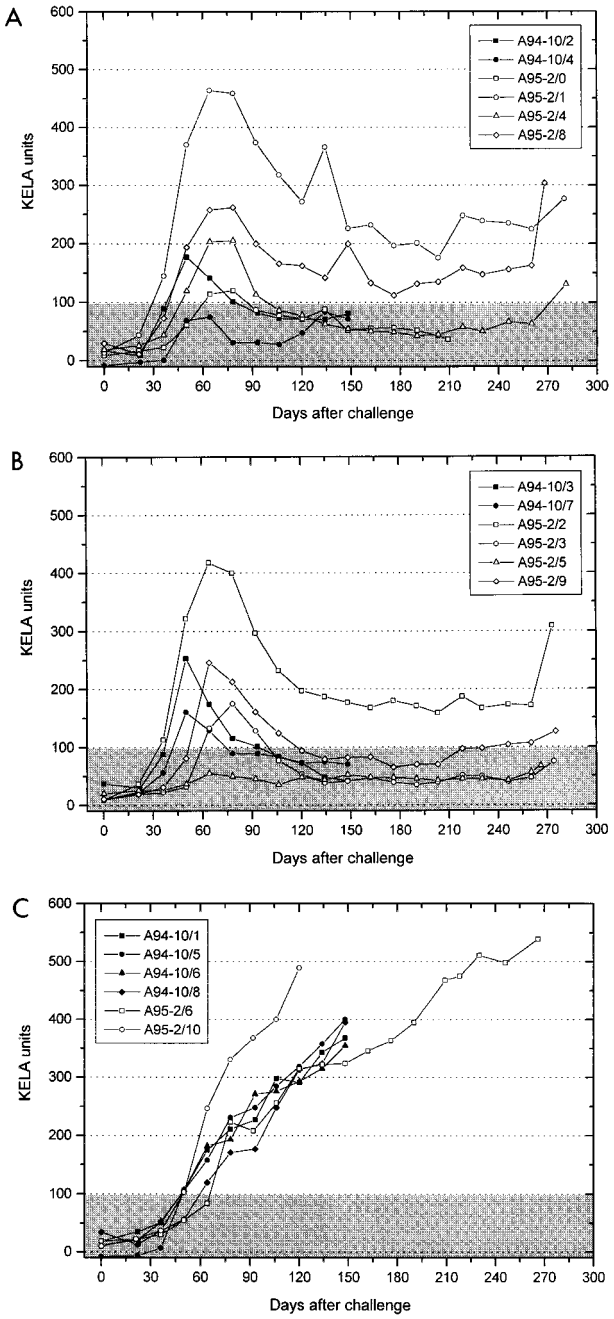

FIG. 2. KELA antibody units to *B. burgdorferi* in sera from dogs exposed to *B. burgdorferi*-infected ticks on day 0. Antibiotic treatment was initiated on day 50 (A94-10 series) and day 69 (A95-2 series). Dogs were euthanized between days 133 and 150 (A94-10 series) and between days 272 and 288 (A95-2 series) after exposure. Note: antibody levels in the shaded area below 100 U are negative. (A) Doxycycline-treated dogs. Note: dog A95-2/0 was euthanized on day 210 after exposure. (B) Amoxicillin-treated dogs. Note: dog A95-2/5 remained uninfected. (C) Untreated infected control dogs. Note: dog A95-2/10 was euthanized on day 120 after exposure.

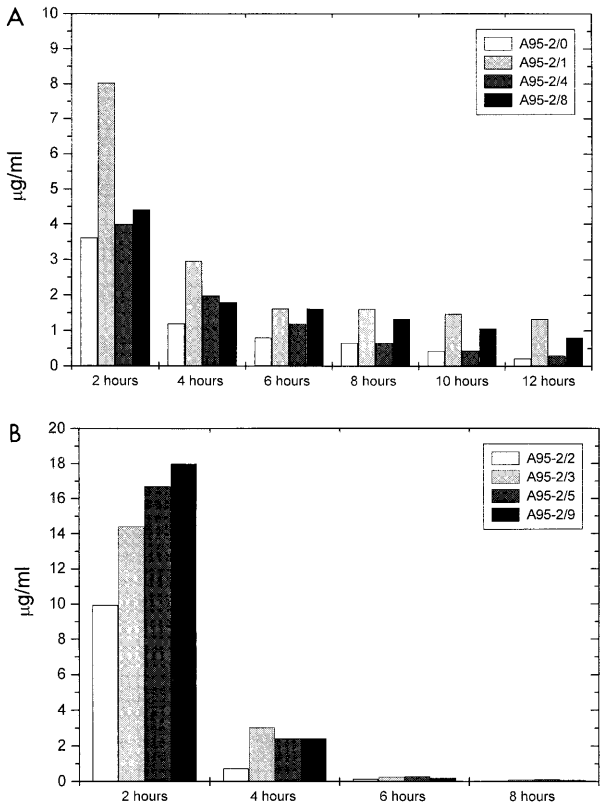

FIG. 3. (A) Doxycycline levels in blood plasma from doxycycline-treated dogs at times posttreatment are shown. Dogs were treated at 12-h intervals. A gel diffusion bioassay using *B. cereus* was used. (B) Amoxicillin levels in blood plasma from amoxicillin-treated dogs at times posttreatment are shown. Dogs were treated at 8-h intervals. A gel diffusion bioassay using *B. stearothermophilus* was used.

30, 37, 45). Similar contradictory results have been obtained in experimental mice (19, 23), hamsters (14, 15), and gerbils (31). Perhaps the time allowed between treatment and testing was not sufficient in most of these studies, since surviving borreliae replicate slowly.

Our results for dogs are in agreement with the results for mice reported by Malawista et al. (19), in which negative culture and PCR were results found by 30 days after termination of treatment but two of six mice were culture positive and one of six mice were PCR positive by 60 days after the last treatment.

We previously developed an experimental model for Lyme disease in the dog (3) in which acute arthritis with lameness as well as persistent *B. burgdorferi* and polyarthritis without clinical signs were documented for up to 1 year after tick exposure. The study was limited to 1 year. In the present study we have addressed the question of whether 4-week treatment with the most commonly used antibiotics for Lyme disease in humans—doxycycline and amoxicillin—is sufficient to eliminate infection and disease. We conclude that antibiotics did ameliorate the disease, as, with one exception, arthritis was not seen in treated dogs. However, *B. burgdorferi*, as judged by culture and PCR, appeared to persist in dogs after 30 days of treatment with either doxycycline or amoxicillin.

B. burgdorferi was isolated from axillary lymph nodes from one amoxicillin- and one doxycycline-treated dog as well as from one skin biopsy sample from a doxycycline-treated dog. However, by PCR using primers for the *B. burgdorferi* 23S rRNA gene and for the outer surface protein A (OspA) gene, we found messages in untreated as well as antibiotic-treated dogs in skin punch biopsy samples and in multiple necropsy tissue samples in three of four doxycycline- and two of three amoxicillin-treated dogs (Table 2; Fig. 1). Tissues from two uninfected SPF dogs and dog 95-2/5, which never became infected, remained PCR negative.

The ultimate proof for persistence of live organisms is isolation. However, Malawista et al. (19) and Persing et al. (28) have shown that spirochetal DNA does not persist in tissues when live borreliae are absent. They suggested that persisting PCR positivity indicates persistent infection. Otherwise, it would be difficult to explain why serum antibody titers initially declined in antibiotic-treated dogs that were kept in isolation but began to increase by 6 months after treatment (Fig. 2B and C). An additional 2- or 3-month waiting period may have produced more positive culture results because of the slow replication of *B. burgdorferi.*

The plasma antibiotic levels in treated dogs were adequate. MICs in doxycycline-treated dogs were maintained throughout the 30-day period of treatment, an important aspect because doxycycline has a bacteriostatic, not bacteriocidal, effect. Because amoxicillin has a bacteriocidal effect, the MICs that lasted for at least 4 h after each treatment should have been sufficient. It is not known whether spirochetes survive antibiotic treatment due to inadequate antibiotic penetration of poorly vascularized connective tissues or whether spirochetes hide intracellularly as was shown in vitro (10, 16, 18). Further, it is unclear whether *B. burgdorferi* develops antibiotic resistance or whether additional factors play a role.

In earlier studies we concluded that spirochete isolation in culture and PCR results were comparable for demonstrating the presence of the infecting agent (3, 7). The PCR in those studies was made with nested primers derived from the *B. burgdorferi* 41-kDa flagellin gene. In our present study, we have used primers for the *B. burgdorferi* 23S rRNA gene and for the OspA gene as reported elsewhere (11, 24, 28, 33). It became obvious that PCR using these primers is more sensitive than isolation of *B. burgdorferi* or nested PCR using 41-kDa primers. While *B. burgdorferi* could only be cultivated from two lymph nodes and one skin biopsy sample from antibiotic-treated dogs, a large number of samples were positive by PCR (Tables 1 and 2).

The persistence of *B. burgdorferi* in mammalian hosts after antibiotic treatment is reminiscent of the persistence of other spirochetes: treponemes and leptospires. It is well known that antibiotic treatment of syphilis patients sometimes fails to eradicate treponemes from the central nervous system and other sites during late syphilis (27). Penicillin treatment is very effective in ameliorating acute symptoms of leptospirosis in humans and animals. However, the infecting agent persists after treatment and kidney failure with uremia is a frequent consequence several months later (1, 47). In contrast, treatment with streptomycin (2, 42) or doxycycline (21) was sufficient to eliminate persistent infection.

In conclusion, the canine model of Lyme disease seems valuable for the detection of persistence of *B. burgdorferi* after exposure to infected ticks and for the response to antibiotic treatment. We have shown that treatment with high doses of amoxicillin or doxycycline for a 30-day period was not sufficient to eliminate the persistent infection. Although (i) the infection rate was greatly reduced, (ii) antibody levels declined after treatment, and (iii) joint lesions were prevented or cured, persistent borreliae stimulated antibody responses again by 6 months after treatment and the possibility of clinical and pathological relapses remained.

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