MAJOR ARTICLE

Xenodiagnosis to Detect *Borrelia burgdorferi* Infection: A First-in-Human Study

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(See the Editorial Commentary by Bockenstedt and Radolf on pages 946–8.)

Background. Animal studies suggest that *Borrelia burgdorferi*, the agent of Lyme disease, may persist after antibiotic therapy and can be detected by various means including xenodiagnosis using the natural tick vector (*Ixodes scapularis*). No convincing evidence exists for the persistence of viable spirochetes after recommended courses of antibiotic therapy in humans. We determined the safety of using *I. scapularis* larvae for the xenodiagnosis of *B. burgdorferi* infection in humans.

Methods. Laboratory-reared larval *I. scapularis* ticks were placed on 36 subjects and allowed to feed to repletion. Ticks were tested for *B. burgdorferi* by polymerase chain reaction (PCR), culture, and/or isothermal amplification followed by PCR and electrospray ionization mass spectroscopy. In addition, attempts were made to infect immunodeficient mice by tick bite or inoculation of tick contents. Xenodiagnosis was repeated in 7 individuals.

Results. Xenodiagnosis was well tolerated with no severe adverse events. The most common adverse event was mild itching at the tick attachment site. Xenodiagnosis was negative in 16 patients with posttreatment Lyme disease syndrome (PTLDS) and/or high C6 antibody levels and in 5 patients after completing antibiotic therapy for erythema migrans. Xenodiagnosis was positive for *B. burgdorferi* DNA in a patient with erythema migrans early during therapy and in a patient with PTLDS. There is insufficient evidence, however, to conclude that viable spirochetes were present in either patient.

Conclusions. Xenodiagnosis using *Ixodes scapularis* larvae was safe and well tolerated. Further studies are needed to determine the sensitivity of xenodiagnosis in patients with Lyme disease and the significance of a positive result.

Clinical Trials Registration. NCT01143558.

Keywords. xenodiagnosis; *Ixodes scapularis*; Lyme disease; *Borrelia burgdorferi*; human.

Lyme disease, caused by *Borrelia burgdorferi* and transmitted by *Ixodes* ticks, is the most common tick-borne illness in the United States and Europe [1]. *Borrelia burgdorferi* enters the skin at the site of the tick bite, typically resulting in the erythema migrans (EM) skin lesion. From the inoculation site, the organism can disseminate and affect the heart, joints, and central nervous system. Antibiotic therapy resolves clinical symptoms in most cases [2].

A minority of patients will have persistent or relapsing nonobjective symptoms (eg, fatigue, musculoskeletal pain, and cognitive complaints, called posttreatment Lyme disease syndrome, [PTLDS]) after receiving a recommended course of antibiotic therapy. The

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pathogenesis of PTLDS remains an area of great controversy [3]. Evidence of ongoing infection has not been found using polymerase chain reaction (PCR) or culture [4, 5]. Current antibody-based assays cannot determine successful eradication of the organism. Randomized placebo-controlled trials have not shown significant, sustained benefit of retreatment with antibiotic therapy in patients with PTLDS [4–6].

Recent animal data suggest that eradication of *B. burgdorferi* by antibiotics may be incomplete. Studies in dogs, mice, and monkeys have shown that *B. burgdorferi* DNA can be detected in tissues for up to 9 months after antibiotic therapy [7–12]. Xenodiagnosis, the use of a vector to detect the presence of an organism, has been used to detect *B. burgdorferi* in animal studies [13–15]. *Ixodes* ticks fed on antibiotic-treated mice and monkeys were able to acquire *B. burgdorferi,* as demonstrated by PCR of the tick and, in some mice studies, transmission to immunodeficient mice during the next blood meal [9–12]. However, some of the animal studies have had methodological concerns [16–18], including infection by needle inoculation instead of tick transmission, use of high-dose inocula of cultured spirochetes, and inability to replicate the antibiotic pharmacokinetics and exposure expected in humans; and, by their very nature, have limited generalizability to human disease.

Here, we present the results of the first study of the use of *I. scapularis* larvae for xenodiagnosis of *B. burgdorferi* infection in humans.

METHODS

Study Protocol

Written informed consent was obtained from all participants. The study was approved by the institutional review board at each center and was conducted in accordance with Good Clinical Practice guidelines and under an investigational device exemption approved by the Food and Drug Administration. An independent medical monitor reviewed interim data for safety.

Participants were enrolled at 3 sites in Massachusetts, Connecticut, and Maryland. All patients were 18 years of age or older. Healthy volunteers resided in endemic areas (Maryland and Massachusetts), had no history of Lyme disease, and were seronegative by C6 antibody enzyme-linked immunosorbent assay (Immunetics, Inc). The patient with EM on treatment (potential positive control) had EM diagnosed by a study physician and started antibiotic therapy concomitantly with tick placement. Patients in the posttreatment EM group had EM diagnosed by a physician and had completed recommended antibiotic therapy [2] between 1 and 4 months before enrollment. Subjects enrolled in the high C6 antibody group had Lyme disease [19], received recommended therapy [2], and had a C6 antibody index >3 for at least 6 months after completion of therapy. Subjects with PTLDS had Lyme disease [19], had received a minimum of 1 course of recommended therapy [2], and had persistent nonspecific symptoms that began coincident with the onset of Lyme disease, which were severe enough to cause a reduction in activities. Subjects in the high C6 antibody and PTLDS groups could not have received antibiotics within 3 months of enrollment.

Study Design

Subjects had 25–30 pathogen-free *I. scapularis* larval ticks (Supplementary Appendix) placed over a 7-cm^2 area under a modified retention dressing (Le Flap, Monarch Labs, Irvine, California; Figure 1).When possible, an area where disease was observed (EM site, close to an affected joint) was selected. Ticks

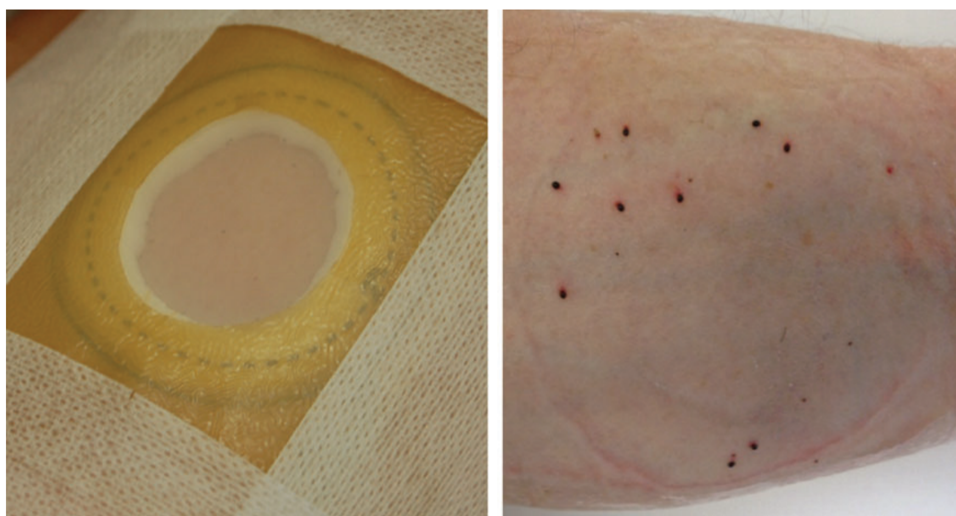

Figure 1. Dressing used for xenodiagnosis. The left panel depicts the LeFlap dressing with unfed ticks placed on the forearm of a subject. The right panel shows the attached feeding ticks.

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were collected 3–7 days after placement. Two 2-mm skin punch biopsies were performed at the exact sites of tick feeding. If <14 engorged ticks were recovered, individuals could repeat the procedure if they still met entry criteria. A repeat procedure was also offered to individuals with a positive xenodiagnostic result.

Participants completed a diary card for the first month, and assessments were done at 7 days, 1 month, and 3 months after tick removal. Adverse events were graded according to the Division of AIDS Table for Grading Adult and Pediatric Adverse Experiences [20].

Testing of Xenodiagnostic Ticks

Two protocols were used for processing of xenodiagnostic ticks. Ticks were tested individually unless noted. In protocol 1, live replete ticks were kept in a humidified chamber. Approximately 3 weeks after molting, nymphal ticks were fed on C3H/HeN severe combined immunodeficiency (SCID) mice. Replete ticks were analyzed for infection by PCR and culture [21, 22] (Supplementary Appendix). SCID mice were monitored for infection by culture and PCR of ear punch biopsies at 2 weeks after tick feeding, and at 4 weeks by culture and PCR of skin, ankle joint, heart, and bladder tissues.

A modified protocol (protocol 2) was used after the 23rd xenodiagnostic procedure. Recovered ticks were kept in a humidified chamber for 11–14 days. Ticks were crushed and tested by PCR and injection of the lysate subcutaneously into an SCID mouse and/or by isothermal amplification followed by PCR and electrospray ionization mass spectroscopy (IA/PCR/ESI-MS) [23–27] (Supplementary Appendix). Skin biopsies were tested by culture and culture PCR. Direct IA/PCR/ESI-MS testing was performed in the skin biopsy of 6 subjects.

Xenodiagnosis was considered positive when any of the techniques demonstrated *B. burgdorferi* or its DNA in a xenodiagnostic tick(s) or in tissue from the SCID mice injected with tick lysate or fed upon by nymphal ticks.

Culture, PCR, and SCID mouse infection were performed at Tufts Medical Center. The investigators were not blinded to participant group status. Testing by IA/PCR/ESI-MS was performed at Ibis Biosciences without knowledge of participant group status.

RESULTS

Subject Characteristics

A total of 36 subjects were enrolled in the study (21 men and 15 women, with a median age of 55 years). Participants who underwent xenodiagnosis included 10 patients with high C6 antibody levels, 10 patients with PTLDS, 5 patients with EM after completion of antibiotic therapy, 1 patient with EM on therapy, and 10 healthy volunteers (Table 1). All patients acquired the infection in the eastern United States. Seven patients underwent >1 xenodiagnostic procedure. These included 5 individuals in the high C6 group, 1 individual in the PTLDS group, and the individual with EM (7 months after completion of antibiotic therapy).

Patients in the high C6 group enrolled a median of 4.5 years after their original diagnosis and received a median of 2.5 courses of antibiotics. The most common presenting manifestation in high C6 patients was Lyme arthritis (Table 1).

Patients in the PTLDS group enrolled a median of 3.8 years after their original diagnosis and received a median of 2 courses of antibiotics. Three of 10 individuals had received intravenous antibiotic therapy. The most common initial presenting manifestation of Lyme disease in PTLDS patients was EM (Table 1). The most common symptoms at enrollment were fatigue, difficulty concentrating, memory complaints, and arthralgias. Eight of the 10 patients still had elevated C6 antibody titers and/or a positive Lyme disease immunoglobulin G (IgG) Western blot (Table 1).

Tick Placement Procedure

As no previous protocol for xenodiagnosis with *I. scapularis* larvae in humans existed, we developed a retention dressing using the Le Flap dressing, modified for use with ticks. Subjects enrolled early in the trial had fewer ticks feed successfully due to entrapment of the ticks in the adhesive. After the addition of a foam ring to create a barrier between ticks and adhesive, we were able to get between 30% and 50% of ticks to feed successfully. Figure 1 shows the dressing used and images before and after xenodiagnosis. Larval ticks required 4–5 days to feed to repletion, (mean, 4.6 days [range, 3–7 days]).

Adverse Events

Xenodiagnosis was well tolerated (Table 2). All subjects successfully completed the tick placement and there were no withdrawals during the study. The most common adverse event was mild itching at the site, which was seen in 58% of subjects, with a median duration of 3 days. Repeat of the xenodiagnosis procedure was similarly well tolerated, with mild itching at the site being the most common complaint. There were no serious adverse events associated with the procedure.

Other adverse events included 10 episodes of mild upper respiratory symptoms, which occurred in 8 individuals. Eight episodes of headaches occurred in 7 subjects. Two episodes were graded as moderate and 1 as severe, and occurred 1–3 months after the procedure. All 7 individuals had a prior history of recurrent headaches.

Testing of Xenodiagnostic Ticks

A total of 162 engorged or partially engorged ticks was recovered from the initial 23 subjects and processed using protocol 1, which included molting and feeding of nymphs on SCID mice. Of these 162 ticks, 80 ticks molted to nymphs, and 77

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Table 1. Participant Demographic Data

ID	Group	US State Where Patient Acquired Infection[a]	Lyme Disease Presentation	Time From Suspected Infection to Therapy, d	Time From Diagnosis to Therapy, d	Time From Diagnosis to Procedure, d	No. of Treatments, IV/Oral	C6 Index	IgG WB	IgM WB	Symptoms Present in the Month Before Enrollment[b]
A-01	EM	MA	EM	0	0	0	0/1	0.5	0/10	1/3	D
B-01	EM PTx	CT	EM	13	0	71	0/1	4.9	1/10	1/3	
B-02	EM PTx	WV	MEM	11	0	96	0/1	4.41	2/10	2/3	D
B-03	EM PTx	WV	MEM	15	0	126	0/1	3.89	3/10	3/3	D
B-04	EM PTx	MD	EM	38	1	115	0/2	5.14	3/10	3/3	
B-05	EM PTx	MA	EM	14	0	138	0/1	1.94	7/10	1/3	B, D
C-02	High C6	VA	EM	1	0	3941	0/7	5.43	2/10	1/3	B, D
C-03	High C6	MD	Arthritis	717	0	1081	0/1	9.57	6/10	0/3	C
C-04	High C6	MD	MEM, FP	24	0	1802	0/1	5.74	4/10	1/3	B, D, I, N
C-05	High C6	MD	FP, meningitis	47	0	714	1/1	4.63	3/10	3/3	F, G
C-06	High C6	VA	EM, arthritis	988	8	894	1/2	8.72	9/10	1/3	B, D, G, K, L, N
C-07	High C6	MD	Arthritis	16	16	1473	0/2	9.42	10/10	1/3	A, B, D, M, N
C-08	High C6	MD	Arthritis	10	1	186	0/1	5.32	7/10	0/3	
C-09	High C6	MD	MEM	12	0	2098	0/3	3.406	5/10	1/3	B, C, D, F, I, K, L, M, N
C-01	High C6	MA or NJ	NB, arthritis	2679	0	2073	1/3	5	7/10	1/3	B, D, M, N
C-10	High C6	MA	EM	0	0	5416	0/3	5	10/10	0/3	
D-01	PTLDS	CT	EM	15	0	6249	1/1	2.31	2/10	2/3	A, B, C, D, E, F, G, H, I, J, K, L, M, N
D-02	PTLDS	MD	Arthritis	23	0	411	0/1	11.04	10/10	2/3	B, D, F, G, H, I, K, L, M, N
D-04	PTLDS	VA	FP	228	0	1721	0/4	4.31	10/10	1/3	B, D, F, M, N
D-03	PTLDS	MA	EM	33	33	2748	0/1	5	7/10	2/3	B, D, M, N
D-05	PTLDS	MA	EM	30	0	256	0/1	5.39	1/10	1/3	D, M, N
D-06	PTLDS	MA	EM	4	0	1050	0/1	0.53	1/10	1/3	D, M, N
D-07	PTLDS	MA	Arthritis	329	0	322	1/2	3.31	9/10	1/3	B
D-08	PTLDS	MA	EM, arthritis	17	10	3068	0/2	5	6/10	1/3	B, D, F, M, N
D-09	PTLDS	PA	EM, arthritis	119	56	438	1/1	0.97	2/10	1/3	B, D, F, G, I, K, L, M, N
D-10	PTLDS	MA	PN	180	0	2247	0/2	5	10/10	1/3	B, D, G, I, K

Abbreviations: C6 index, IgG and IgM Western blot results at study entry; EM, erythema migrans; EM PTx, after completing antibiotic therapy for erythema migrans; FP, facial palsy; IgG, immunoglobulin G; IgM, immunoglobulin M; IV, intravenous; MEM, multiple erythema migrans; NB, neuroborreliosis; PN, peripheral neuropathy; PTLDS, posttreatment Lyme disease syndrome; WB, Western blot.

[a] CT, Connecticut; MA, Massachusetts; MD, Maryland; NJ, New Jersey; PA, Pennsylvania; VA, Virginia; WV, West Virginia.

[b] A, decreased appetite; B, arthralgia; C, cough; D, fatigue; E, fevers; F, headaches; G, stiff neck; H, abdominal pain; I, myalgia; J, nausea; K, tingling; L, numbness; M, difficulty concentrating; N, memory problems.

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Table 2. Adverse Events

Adverse Event	No. of Episodes
Itching at the xenodiagnosis site	27 episodes in 21 subjects
Itching related to the dressing	6 episodes in 6 subjects
Itching to skin punch biopsy site	4 episodes in 3 subjects
Erythema at the skin punch biopsy site	2 episodes in 2 subjects
Upper respiratory infection	10 episodes in 8 subjects
Headache	8 episodes in 7 subjects
Mild nausea without apparent cause	3 episodes in 3 subjects

Adverse events that occurred in >1 subject are shown.

nymphs were placed on SCID mice. Only 37 nymphs were recovered after feeding on SCID mice (Table 3). Four participants had no fed or partially fed ticks recovered, and 7 participants had no xenodiagnostic ticks tested due to loss during molting or feeding on the SCID mice.

Due to the loss of ticks during the molting and feeding on the SCID mice, the protocol for processing recovered ticks was changed. For protocol 2, all ticks were tested after a 10- to 14-day incubation to allow replication of any acquired *B. burgdorferi*. Ticks were tested by (1) culture, PCR, injection of lysates into SCID mice with subsequent culture and PCR and/or (2) IA/PCR/ESI-MS. From 13 subjects, 146 ticks were tested by PCR and culture; and from 14 individuals, 178 ticks were tested directly by IA/PCR/ESI-MS. For 7 individuals, ticks were tested by both PCR and culture and IA/PCR/ESI-MS (Table 3).

Ticks from healthy volunteers tested by protocol 1 and 2 were negative (Tables 3 and 4). Twenty-three subjects with Lyme disease had at least 1 tick tested by protocol 1 and/or 2 (Table 3). From these 23 participants, 19 tested negative. There were indeterminate results in 2 patients, attributed to laboratory contamination. All tissues from SCID mice that had been fed upon by nymphs (protocol 1) or were injected with lysate from recovered ticks (protocol 2) tested negative by PCR and culture (Table 4).

Results from subject A-01, EM on therapy, were considered positive by IA/PCR/ESI-MS on 2 separate specimens: 1 from a single tick and 1 from a pool of 3 ticks. The single tick was positive for 6 of 8 primer pairs, with primer pair BCT3519 [25] producing 2 *Borrelia* amplicons, indicating that this tick contained a mixture of 2 *B. burgdorferi* genotypes. The tested pooled specimen was positive with 2 of 8 primer pairs and the base-count signatures detected matched the signatures found in the single tick. Six other ticks from this subject tested negative by culture and PCR. The skin biopsy was negative by culture and culture PCR. This participant was completing the fourth day of antibiotic therapy when the ticks were collected and the skin biopsy was performed. This individual repeated the xenodiagnostic procedure 7 months after completing antibiotic therapy, and IA/PCR/ESI-MS testing of 10 recovered ticks was negative (Table 3).

One subject with PTLDS (D-02) was considered positive in 2 separate xenodiagnostic procedures. Ticks recovered from the initial xenodiagnostic procedure were tested using protocol 1. One nymph was found to be positive by PCR of the nymph lysate culture, but direct PCR of the nymph lysate and microscopic evaluation of the culture were negative. Four other nymphs were negative in all testing. All tissues from the SCID mouse on which the nymphs were fed were negative (including the nymph associated with the positive PCR assay). The patient's skin biopsy, performed at the site of the tick feeding, was negative by culture and culture PCR. The original positive *ospA* PCR of the tick culture was confirmed by PCRs for other *B. burgdorferi* genes. PCR for *flaB*, *ospC*, and a second primer set for *ospA* were positive, but *recA* PCR was negative. The DNA extracted from this culture sample was then tested by IA/PCR/ESI-MS, which was positive for 7 of the 8 assay primer pairs. It identified the DNA as from a novel genotype of *B. burgdorferi*, due to its unique combination of base-count signatures [25]. All strains of *B. burgdorferi* in use in the Tufts University laboratory were characterized by the IA/PCR/ESI-MS assay and none of the strains matched this genotype, making the possibility of contamination unlikely. Xenodiagnosis was repeated approximately 8 months after the original procedure. Direct testing by IA/PCR/ESI-MS revealed that 1 tick was positive for *B. burgdorferi*, by detection of 2 of 8 assay primer pairs. The 2 base-count signatures detected were consistent with the previously found genotype. Another tick was tested by PCR and culture and was negative. The patient's 2 repeat skin biopsy samples were negative by culture and culture PCR.

Skin Biopsy Testing

All but 6 participants had skin biopsies performed at the site of tick feeding on completion of the xenodiagnostic procedure. Twenty-nine samples were negative by culture and culture PCR. These included the biopsies from the 2 participants with positive results on the xenodiagnostic testing. Six samples (not including the 2 positive cases) were tested directly by IA/PCR/ESI-MS and were negative.

DISCUSSION

The primary goals of this study were to develop procedures for xenodiagnostic testing of patients with Lyme disease and to determine the safety of tick xenodiagnosis in humans. We demonstrated that up to 30 larval ticks can be safely applied to humans. Adverse events were minimal and limited predominantly to itching at the tick bite sites.

Our initial results show that the majority of the patients with Lyme disease treated with antibiotic therapy are negative by xenodiagnosis. An important caveat is that the number of tested

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Table 3. Results of Xenodiagnostic Testing

Group	ID	Protocol	No. of Fed Ticks Tested by PCR/Culture	No. of Positive Ticks	No. of Fed and Partially Fed Larvae Tested by IA/PCR/ESI-MS	No. of IA/PCR/ESI-MS Positive Results
EM	A-01	2	6	0	4	2
EM PTx[a]	A-01R	2	ND	ND	10	0
EM PTx	B-01	1	0	N/A	N/A	N/A
EM PTx	B-02	1	1	0	N/A	N/A
EM PTx	B-03	1	2	0	N/A	N/A
EM PTx	B-04	1	8	0	N/A	N/A
EM PTx	B-05	2	11	0	11	0
High C6	C-01	1	0	N/A	N/A	N/A
High C6	C-02	1	1	0	N/A	N/A
High C6	C-02R	2	ND	ND	21	0
High C6	C-03	1	1	0	N/A	N/A
High C6	C-03R	2	ND	ND	9	0
High C6	C-04	1	5	0	N/A	N/A
High C6	C-04R	2	ND	ND	24	0
High C6	C-05	1	1	0	N/A	N/A
High C6	C-06	1	0	N/A	N/A	N/A
High C6	C-06R	2	ND	ND	23	0
High C6	C-07	1	0	N/A	N/A	N/A
High C6	C-07R	2	ND	ND	20	0
High C6	C-08	2	8	0	ND	ND
High C6	C-09	2	19	0	ND	ND
High C6	C-10	2	9	0	ND	ND
PTLDS	D-01	1	0	N/A	N/A	N/A
PTLDS	D-02	1	5	1	N/A	N/A
PTLDS	D-02R	2	1	0	1	1
PTLDS	D-03	1	0	N/A	N/A	N/A
PTLDS	D-04	1	7	0	N/A	N/A
PTLDS	D-05	2	22	0	ND	ND
PTLDS	D-06	2	24	0	ND	ND
PTLDS	D-07	2	15	0	ND	ND
PTLDS	D-08	2	7	0	5	0
PTLDS	D-09	2	ND	ND	24	0
PTLDS	D-10	2	5	0	5	0
HV	E-01	1	0	N/A	N/A	N/A
HV	E-02	1	1	0	N/A	N/A
HV	E-03	1	0	N/A	N/A	N/A
HV	E-04	1	3	0	N/A	N/A
HV	E-05	1	0	N/A	N/A	N/A
HV	E-06	1	0	N/A	N/A	N/A
HV	E-07	1	2	0	N/A	N/A
HV	E-08	1	0	N/A	N/A	N/A
HV	E-09	2	8	0	10	0
HV	E-10	2	11	0	11	0

Abbreviations: EM, erythema migrans; EM PTx, after completing antibiotic therapy for erythema migrans; High C6, subjects with persistently elevated C6 antibody serum levels; HV, healthy volunteers; IA/PCR/ESI-MS, isothermal amplification followed by polymerase chain reaction and electrospray ionization mass spectroscopy; N/A, not applicable; ND, not done; PCR, polymerase chain reaction; PTLDS, posttreatment Lyme disease syndrome; R, repeat xenodiagnostic procedure.

[a] Procedure was repeated 7 months after completing antibiotic therapy and the patient was asymptomatic. Nymphal ticks were tested by PCR and culture in protocol 1; larval ticks were tested in protocol 2. Direct testing of ticks by IA/PCR/ESI-MS was performed only under protocol 2. Individual ticks were evaluated by only 1 of the 2 methods.

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Table 4. Results of Severe Combined Immunodeficiency Mice Testing

Group	ID	Protocol	No. of Nymphal Ticks Applied to Mice	No. of Fed and Partially Fed Larvae Used in the Lysate	SCID Organ Culture and PCR Result
EM	A-01	2	N/A	6	NEG
EM PTx	B-02	1	1	N/A	NEG
EM PTx	B-03	1	3	N/A	NEG
EM PTx	B-04	1	13	N/A	NEG
EM PTx	B-05	2	N/A	11	NEG
High C6	C-02	1	5	N/A	NEG
High C6	C-03	1	1	N/A	NEG
High C6	C-04	1	10	N/A	NEG
High C6	C-05	1	4	N/A	NEG
High C6	C-06	1	9	N/A	NEG
High C6	C-07	1	8	N/A	NEG
High C6	C-08	2	N/A	8	NEG
High C6	C-09	2	N/A	19	NEG
High C6	C-10	2	N/A	9	NEG
PTLDS	D-02	1	6	N/A	NEG
PTLDS	D-02R	2	N/A	1	NEG
PTLDS	D-04	1	10	N/A	NEG
PTLDS	D-05	2	N/A	22	NEG
PTLDS	D-06	2	N/A	24	NEG
PTLDS	D-07	2	N/A	15	NEG
PTLDS	D-08	2	N/A	7	NEG
PTLDS	D-10	2	N/A	5	NEG
HV	E-02	1	1	N/A	NEG
HV	E-04	1	4	N/A	NEG
HV	E-07	1	2	N/A	NEG
HV	E-09	2	N/A	8	NEG
HV	E-10	2	N/A	11	NEG

Abbreviations: EM, erythema migrans; EM PTx, patients after completing antibiotic therapy for erythema migrans; High C6, subjects with persistently elevated C6 antibody serum levels; HV, healthy volunteer; N/A, not applicable; NEG, negative; PCR, polymerase chain reaction; PTLDS, posttreatment Lyme disease syndrome; R, repeat xenodiagnostic procedure; SCID, severe combined immunodeficiency.

xenodiagnostic ticks per participant in general was small, particularly in the early subjects. The number of engorged ticks tested for each individual is likely to be an important variable. The more engorged ticks tested, the larger the probability of detecting a positive. However, our testing of the ticks included multiple modalities and very sensitive assays targeting *B. burgdorferi*.

We found the presence of amplifiable *B. burgdorferi* DNA in xenodiagnostic ticks removed from 2 individuals. One individual had EM, and had just started antibiotic therapy. The aim in evaluating this participant was to include a possible "positive control" for the xenodiagnostic procedure in humans. Although the best positive controls would be individuals with untreated EM, we felt it would be inappropriate to withhold therapy for the few days required for tick feeding, due to the risk of dissemination of the organism and possible morbidity. The second individual was a patient with PTLDS, who had *B. burgdorferi* DNA detected from 2 different xenodiagnostic procedures, 8 months apart. We were unable to culture the organism or to show transmission to SCID mice.

All of our positive detections were only through DNA amplification techniques. Although this is consistent with the animal studies of xenodiagnosis after antibiotics [9–12], where positivity as assessed by PCR is at very low levels and not associated with identifiable pathology in the animal, it raises the question of whether detection represents the presence of viable organisms. The inability to culture the organism may be due to genetic loss of plasmids by *B. burgdorferi* [9]. In studies in which *B. burgdorferi* infection was introduced into mice using cultured spirochetes, xenodiagnosis performed after antibiotic treatment suggested that spirochetes detectable in ticks by PCR but not by culture were viable, because ticks could transmit the infection to SCID mice [10, 11]. Transmission was unsuccessful, however, in other mice studies in which infection was introduced using ticks, which models more closely human acquisition of Lyme disease [9, 28]. If active motility were required for the acquisition of *B. burgdorferi* by ticks, a positive xenodiagnostic test would imply viability. In the "positive control," viable spirochetes could have been killed or inhibited by antibiotics in the skin as well as in the blood and interstitial fluid ingested by the tick during its 4-day feeding period [28, 29]. However, an alternative hypothesis is that ticks acquire DNA from dead organisms during their feeding. *Borrelia burgdorferi* was not found in skin biopsies at the tick feeding sites, but the majority of the samples were tested using culture. IA/PCR/ESI-MS testing of additional skin biopsies might help to clarify whether spirochetal DNA can also be detected directly in skin. A study of patients with Lyme arthritis has showed that *B. burgdorferi* DNA can persist in the synovial fluid and may not be a marker of viability of the bacteria [30].

Limitations of this study include the relatively small number of patients on which xenodiagnosis was attempted. However, the majority of patients studied were those with residual nonspecific symptoms and persistent seropositivity, which is the group of greatest interest with regard to the question of whether there might be residual infection with *B. burgdorferi*. Also, because of the nature of patient recruitment, monitoring of compliance with antibiotic therapy for the prior episode of Lyme disease or documentation of the adequacy of blood levels of the antibiotics prescribed was not performed. We cannot exclude the possibility of untreated subclinical reinfections that might have occurred after completion of the last known course of antibiotic treatment; however, it should be noted that reinfected patients would be missed by currently available testing.

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This study establishes that xenodiagnosis can safely be used as a tool in patients with Lyme disease and has potential for furthering our knowledge of *Borrelia* biology in humans. Future studies are necessary to determine the incidence of positive xenodiagnostic results for *B. burgdorferi* after antibiotic treatment, if these results represent viable organisms or remnants of infection, and whether these results can be related to ongoing symptoms in patients after therapy for Lyme disease.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. M. W. E., C. D. C., and H. E. C. are employees of Ibis Biosciences, an Abbott company, which developed the PCR/ESI-MS and IA/PCR/ESI-MS assays and instrumentation used in these studies. S. R. T. has received consulting fees from Immunetics and is a shareholder of Abbott. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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