

Atovaquone Plus Cholestyramine in Patients Coinfected With *Babesia microti* and *Borrelia burgdorferi* Refractory to Other Treatment

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ABSTRACT

Ten percent of US patients with Lyme disease are coinfecting with *Babesia microti*. A double-blind, placebo-controlled, crossover trial enrolled 25 patients with confirmed *Borrelia burgdorferi*/*B. microti* coinfection, abnormal visual contrast sensitivity (VCS), and persistent symptoms despite prior treatment with atovaquone and azithromycin. Patients were randomly assigned to atovaquone suspension or placebo plus cholestyramine for 3 weeks, were crossed over for 3 weeks, and then received open-label atovaquone and cholestyramine for 6 weeks. Symptoms and VCS scores were recorded at baseline and after weeks 3, 6, 9, and 12. Improvements in symptoms and VCS deficits were observed only after at least 9 weeks of treatment. At week 12, 5 patients were asymptomatic, and 16 had a notable reduction in the number of symptoms. The entire cohort demonstrated significant increases in VCS scores. Adverse effects were rare. Patients coinfecting with *B. burgdorferi* and *B. microti* derive measurable clinical benefit from prolonged treatment with atovaquone and cholestyramine. Longer-term combination therapy may be indicated.

Keywords: babesiosis; Lyme disease; atovaquone; cholestyramine; visual contrast sensitivity

INTRODUCTION

Lyme disease continues to be a significant vector-borne infectious disease in the United States, with nearly 24,000 new cases reported to the Centers for Disease Control and Prevention (CDC) in 2002 and more than 157,000 cases documented since 1982.^{1,2} Most US cases occur in the wooded and grassy regions of the Northeast, where ixodid ticks infected with the spirochete *Borrelia burgdorferi* feed on deer, dogs, humans, and mice. The same vector is responsible for infections caused by the apicomplexan intraerythrocytic parasite *Babesia microti*. Reports of *Borrelia/Babesia* coinfection are multiplying as more people move into wooded areas to live, thereby increasing their exposure to infected ticks. Deer populations are on the rise, and reports of babesiosis suggest that the organism is expanding its endemic range from coastal areas inland and farther south. In Connecticut, 11% of people with Lyme disease are also infected with *Babesia*, and in the northern Midwest, coinfection has been observed in 9% to 16% of patients with Lyme disease.^{3,4} Coinfection, which is generally manifested by more severe symptoms than occur with either disease by itself, represents a major therapeutic challenge.⁴

Patients with Lyme disease are usually treated with a 21-day course of doxycycline, amoxicillin, or cefuroxime axetil^{5,6} that often relieves the flulike symptoms. Up to 60% of treated patients, however, eventually experience arthritic symptoms or a post-Lyme syndrome (PLS) of diffuse arthralgia, myalgia, fatigue, and cognitive dysfunction that is usually not amenable to antibiotic therapy.⁷⁻¹⁰ Recent evidence suggests that PLS symptoms may be related to a neurotoxin produced by *Borrelia*.¹¹⁻¹³ Babesiosis is usually managed with azithromycin plus atovaquone suspension or quinine plus clindamycin for 7 to 10 days, or by exchange transfusion as a supplement in severe cases.^{14,15} In 1 study of 58 patients with babesiosis,¹⁶ 7 days of treatment with atovaquone (750 mg every 12 hours) plus azithromycin (500 mg on day 1; 250 mg daily thereafter) was as effective as, but better tolerated than, clindamycin (600 mg every 8 hours) plus quinine (650 mg every 8 hours). A cohort of patients in this study also had persistent symptoms after therapy had ended.

It is not known whether *Babesia* secretes a neurotoxin. A recent study demonstrating production of a neurotoxin by *Plasmodium falciparum*¹⁷ and a veterinary trial¹⁸ showing successful results of neurotoxin binding in horses coinfecting with *B burgdorferi* and *Sarcocystis*, an apicomplexan genus closely related to *Babesia*, support the hypothesis that *Babesia* may also make neurotoxins. No studies have assessed the possibility of deficits in visual contrast sensitivity (VCS) in *Babesia*-infected patients. VCS testing with a noninvasive, portable device (Functional Acuity Contrast Test [FACT®]; Stereo Optical Co, Chicago, Ill) has uncovered deficits in patients with acute and chronic illnesses acquired after exposure to toxigenic dinoflagellates and water-damaged buildings with resident toxigenic organisms, including fungi, but not in patients who remained unexposed to biologically produced neurotoxins. VCS deficits have also been investigated in patients with Lyme disease.^{11,19-21}

Novel pharmacologic approaches to the treatment of patients with PLS have been investigated.^{22,23} Cholestyramine, a nonabsorbable polymer that binds salts from bile through anion exchange, has been used at doses approved for the treatment of hypercholesterolemia to bind to neurotoxins in patients with Lyme disease.¹¹ In a study of 111 patients with PLS,²¹ cholestyramine significantly reduced symptoms and improved abnormally low mid-spatial frequency deficits in VCS. Early in treatment, however, 53% of patients experienced significant intensification of symptoms—a phenomenon not seen with other biotoxin exposures. Pretreatment of 125 subsequent patients with PLS using pioglitazone, a peroxisome proliferator-activated receptor agonist, reduced this reaction by 96%; plasma levels of the proinflammatory cytokine, tumor necrosis factor- α , were also reduced.¹¹ In another trial,²¹ 241 patients with PLS who remained symptomatic despite antibiotic therapy received pioglitazone 45 mg daily for 10 days. Cholestyramine was begun on day 6 of this regimen and was continued until maximum symptom abatement and maximum improvement in VCS scores were rated (21–60 days). No antibiotics or other interventions were used during the study. With the pioglitazone/cholestyramine regimen, 92% of patients had at least a 50% reduction in symptoms; 83% had a reduction of 75%; 23% noted complete resolution; and 3.4% showed no improvement. VCS deficits before treatment and resolution with treatment were better correlated with symptom abatement than were other diagnostic markers. Symptoms intensified in only 2% of patients. No adverse events related to pioglitazone (eg, hypoglycemia, abnormal liver function values) were reported. An independent study in Germany confirmed these results.²⁴

The present study investigated the efficacy of combined toxin-binding therapy with cholestyramine and atovaquone, but without pioglitazone or other antibiotics, in patients coinfecting with *B microti* and *B burgdorferi* whose symptoms had not been reduced with atovaquone/azithromycin. The primary hypothesis was that such nonresponsive coinfecting patients had an illness caused by biologically produced neurotoxins, whose eradication would be necessary for clinical improvement. The secondary hypothesis was that the *B microti* neurotoxin prevented clinical improvement compared with that seen in non-coinfecting patients with PLS, and that Lyme coinfection prevented eradication of *B microti* with short courses of atovaquone and azithromycin. VCS scores and improvement in symptoms were used to assess response in a manner similar to that used in the investigators' earlier studies in patients with illnesses caused by Lyme ticks, dinoflagellates, and fungi.

PATIENTS AND METHODS

Patients

Patients who came to a single medical clinic for treatment of persistent illness after a tick bite were offered enrollment in this trial if comprehensive evaluation revealed case-defined simultaneous babesiosis and Lyme disease without confounding illnesses (Table 1). Study medications were provided at no charge, but no other financial incentives were offered.

Table 1. Case Definition

1. Potential for exposure to endemic areas for babesiosis and Lyme disease, with a single illness acquired after a tick bite
2. Multiple symptoms from multiple organ systems
3. Clinical evidence of a simultaneously acquired coinfection and not 2 separately acquired infections
4. Positive results for both organisms on tests performed by standard laboratories
5. No evidence of any confounding illnesses or exposure to biologically produced neurotoxins from water-damaged buildings or estuaries
6. Previous unsuccessful treatment with both appropriate antibiotics and a toxin-binding protocol

The following inclusion criteria applied: patient at least 18 years old; confirmed diagnostic tests (smear or polymerase chain reaction) for both *B microti* and *B burgdorferi* (Western blot); residence in or travel to areas with endemic populations for Lyme disease and *B microti*; no illness suggestive of either Lyme disease or babesiosis before onset of the acute illness; symptoms compatible with babesiosis (irregular fever, chills, headache, diaphoresis, myalgia, fatigue) and Lyme disease (erythema migrans rash; flulike illness with fever, chills, and myalgia; headache or stiff neck; arthralgias; arthritis); persistent symptoms (>6 from a standard list) after 3 or more weeks of treatment with doxycycline, amoxicillin, or cefuroxime axetil, followed by 3 or more weeks of azithromycin plus atovaquone, followed by at least 1 month of pioglitazone plus cholestyramine toxin-binding therapy; a VCS deficit; visual acuity at least 20/50; and no confounding exposures to biologically produced neurotoxins.

Exclusion criteria consisted of participation in other investigational drug protocols within 28 days before screening; a diagnosis of ehrlichiosis or allergy to atovaquone or cholestyramine; abnormal liver function values or phenylketonuria; treatment with rifampin or rifabutin within 28 days of study entry or at any time during the study; history of chronic solvent exposure, chronic alcoholism, or occupational exposure to metal fumes, dust, or petroleum products; acute illness separate from Lyme/*Babesia* coinfection that required immediate treatment; and diagnosis of complete biliary obstruction.

No medications other than study drugs were prescribed. Nineteen of the 25 patients had received more than 3 courses of atovaquone/azithromycin; only 1 patient had been treated for 3 weeks. At some point before study entry, 24 patients had received more than 3 weeks of oral antibiotics for Lyme disease, and 17 had been treated with intravenous antibiotics for at least 4 weeks. On at least 1 occasion, all patients were given adequate therapy for both Lyme and *Babesia* simultaneously. No correlation was noted between prior therapies and symptoms or VCS before the study began.

Study Design

This double-blind, placebo-controlled, crossover study was conducted at the Center for Research on Biotoxin-Associated Illnesses, in Pocomoke City, Maryland, from February 19 through October 31, 2001. The protocol was reviewed and approved by the center's institutional review board and the US Food and Drug Administration. All patients provided informed consent before enrollment.

Patients were randomly assigned to treatment with atovaquone suspension 45 mL (750 mg) twice daily or matched placebo for 3 weeks, in combination with cholestyramine 9 g 4 times daily; they were then crossed over for 3 weeks. Atovaquone was supplied as Mepron® Suspension (GlaxoSmithKline, Research Triangle Park, NC) and cholestyramine as Questran® (Bristol-Myers Squibb, Princeton, NJ). Atovaquone was given at least 1 hour before or after administration of cholestyramine to avoid possible cholestyramine binding (and reduced bioavailability) of atovaquone. This 6-week period was followed by open-label treatment with both medications for 6 additional weeks.

Primary endpoints consisted of subjective clinical improvement, measured by reduction in the number and severity of symptoms, and objective improvement (increases) in VCS scores. Targeted symptoms and VCS scores were recorded at baseline and every 3 weeks thereafter through week 12. The FACT device was used to measure VCS scores. Secondary endpoints included safety and tolerability of treatment. So that adverse events could be reported, participants were asked open-ended questions about how they felt at each study visit.

Statistical Analysis

Descriptive statistics were applied to changes in symptoms. Changes from baseline in VCS scores were analyzed by means of multivariate analyses of variance (MANOVA, with Wilks' λ statistic) suitable for a repeated-measures test. *P* values <.05 were deemed statistically significant.

RESULTS

Population

Of 25 enrolled patients, 21 completed the study. Noncompletion was due to cholestyramine intolerance (2), lack of improvement at week 9 (1), or reasons unrelated to the study (1). All completers were white; 15 (71%) were female. Patient mean age was 45.4 years (range, 30–54 y). At baseline, no differences were observed in number or severity of symptoms and VCS deficits between the placebo/cholestyramine group and the atovaquone/cholestyramine group. Cognitive function, measured with a symptom list used for patients with illnesses caused by other toxigenic organisms, did not differ between groups; neither did diagnostic test results used to confirm *Babesia*/Lyme coinfection.

Efficacy

No notable symptom resolution or improvement in VCS scores occurred during the 6-week double-blind period or the subsequent 2 weeks of the open-label period. Nine weeks into the trial, however, most patients reported significant improvement in symptoms. At week 12, 16 patients noted at least a 50% reduction in symptoms, and 5 patients reported no symptoms or just 1 symptom. The most demonstrable improvement occurred in targeted symptoms related to cognitive dysfunction (eg, concentration difficulty, memory problem, confusion, disorientation), with moderate improvement in muscle ache, joint ache, and headache, and lesser improvement in fatigue (Table 2). The percentage of patients with more than 12 symptoms decreased from 27% at baseline to 0% at week 12 (Table 3). Week 12 VCS scores were significantly improved ($P<.05$) from baseline (Figure). No relapses were recorded. No significant reduction in cholesterol levels was noted during this short-term treatment with cholestyramine.

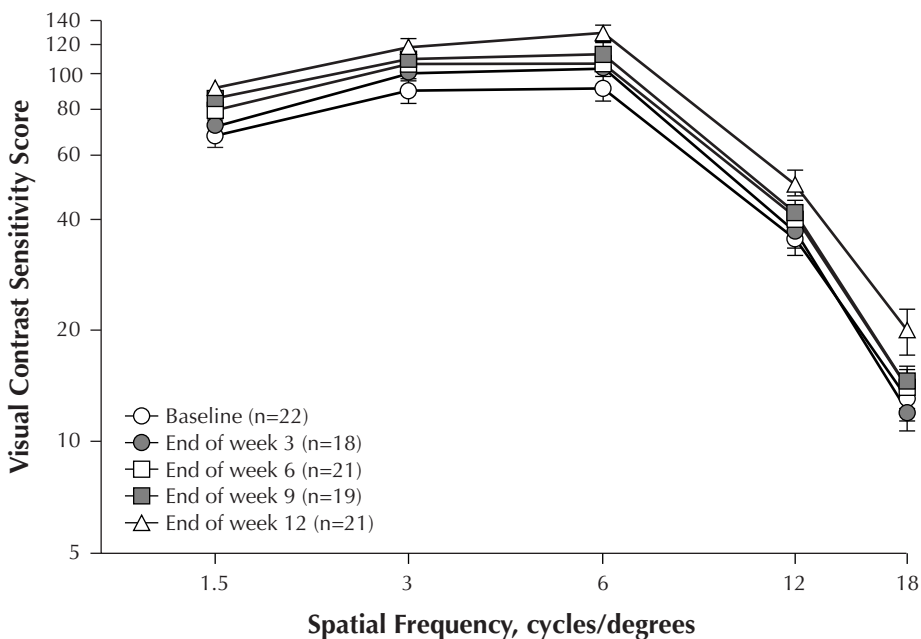
Table 2. Targeted Symptoms Monitored During the Study

Symptom	Baseline	Week 3	Week 6	Week 9	Week 12
Fatigue	77	83	84	66	68
Muscle ache	77	55	68	47	36
Joint pain	68	66	57	52	40
Headache	54	55	52	42	27
Memory problem	54	44	52	28	13
Light sensitivity	45	40	31	28	9
Weakness	45	40	57	28	22
Concentration difficulty	45	44	36	23	4
Cramp	40	27	31	33	9
Shortness of breath	40	33	21	19	4
Numbness	36	33	21	4	4
Tingling	31	33	21	23	9
Sinus problems	31	27	15	14	4
Tearing	27	22	15	9	4
Confusion	22	22	21	9	4
Metallic taste	22	16	15	14	4
Skin problem	22	16	10	14	4
Unusual pain	22	0	0	0	0
Cough	22	22	15	9	0
Disorientation	18	11	5	4	0
Vertigo	18	22	15	9	0
Abdominal pain	13	11	15	4	4
Blurred vision	13	16	10	0	0
Red eyes	13	5	5	0	0
Diarrhea	4	5	5	0	0

Table 3. Patients With Symptoms at Each Stage of the Study

Stage	Patients, n	Symptoms/Patient, n						
		0-1	2-3	4-5	6-7	8-9	10-11	≥12
Baseline	22	0	4	9	36	13	9	27
Week 3	19	0	10	21	14	21	21	10
Week 6	19	0	10	31	21	15	14	5
Week 9	21	4	19	42	14	9	4	4
Week 12	22	27	40	27	0	0	4	0

Visual contrast sensitivity scores during the study.



Safety

A slight worsening of symptoms (primarily fatigue) was noted in some patients prior to improvement. Gastrointestinal adverse events generally decreased during the study; this could not be attributed solely to either atovaquone or cholestyramine.

DISCUSSION

Results of this study indicate that at least 9 weeks of treatment with atovaquone plus cholestyramine is required to relieve symptoms and improve VCS in patients with antibiotic-resistant Lyme/babesiosis coinfection. Because the first 6 weeks of this study used a double-blind, placebo-crossover design, half the patients were given atovaquone during the first 3 weeks and placebo during the second 3 weeks (followed by 6 weeks of open-label atovaquone). The week 12 response of these patients did not differ from that of patients who received placebo during the first 3 weeks, followed by 9 continuous weeks of atovaquone. Thus, although it is possible that the critical factor in managing Lyme/babesiosis coinfection with atovaquone/cholestyramine is at least 6 weeks of uninterrupted treatment, the total duration of therapy appears to be more important.

In some patients, fatigue worsened slightly (by 6%–7%) before it improved. Incidences of joint pain, headache, vertigo, blurred vision, abdominal pain, and cough remained essentially unchanged between baseline and the end of week 3. Worsening of symptoms before improvement has been reported in patients with Lyme disease and can be prevented by initiation of pioglitazone a few days prior to use of cholestyramine.¹¹ Such temporary intensification of symptoms may have been related to a Jarisch-Herxheimer-like reaction, with increased serum levels of tumor necrosis factor or matrix metalloproteinase^{9^{11,25}}; this occurs in approximately 15% of patients with early disseminated infection.²⁶ Although discontinuation of azithromycin before this study began may have prompted a resurgence of certain babesiosis symptoms, it may also have prompted gastrointestinal tolerance of treatment owing to the absence of azithromycin-related nausea, abdominal pain, and diarrhea or loose stools. No patient reported constipation—the primary adverse event seen with cholestyramine.

The use of cholestyramine in this study was unique in that the product labeling for this anion-binding resin lacks this indication.²⁷ The authors of this study believe, however, that cholestyramine is likely responsible for alleviating or lessening the severity of PLS symptoms through its binding to putative *Borrelia*-secreted toxins and elimination of them from the body in the feces. In a double-blind, placebo-controlled clinical trial that used VCS measurements,²⁸ cholestyramine reduced symptoms of a chronic neurotoxin-associated illness caused by *Pfiesteria piscicida* that met all CDC criteria for possible estuary-associated syndrome (PEAS). Two other studies^{29,30} support the use of therapy with cholestyramine and the diagnostic use of VCS in patients exposed to water-damaged buildings with resident toxin-forming genera of fungi, so-called sick building syndrome. The same benefits of cholestyramine and VCS testing occurred in patients who became ill after consuming reef-dwelling fish contaminated with neurotoxins made by ciguatera.³¹ Additional advantages associated with the use of cholestyramine to reduce VCS were reported in a small cohort of patients with a neurotoxin-associated illness caused by the cyanobacterium *Cylindrospermopsis*.³² Cholestyramine is a valuable adjunct to vancomycin in the treatment of patients with *Clostridium difficile*-related colitis; it binds toxins A and B produced by that organism.³³⁻³⁵

VCS scores were used to evaluate improvement because they have correlated with abatement of clinical symptoms during cholestyramine treatment of neurotoxin-related PEAS,^{28,36} sick building syndrome,^{29,30} and cyanobacterial and dinoflagellate

illnesses.^{28,31,32,36-38} An early study of patients with antibiotic-resistant chronic Lyme disease showed that more than 92% had VCS deficits, which were reversed in 98% of responders to cholestyramine and pioglitazone.¹¹ Findings of clinical improvement and reduced VCS deficits in the study population would support the hypothesis that *Babesia* organisms also make a neurotoxin. Mechanisms responsible for the VCS deficit and its correction by cholestyramine have not been delineated.

The atovaquone/cholestyramine regimen in this study was especially effective in relieving post-Lyme disease cognitive/neurologic symptoms reported by a high proportion of patients at baseline. In previous studies,⁷⁻¹⁰ these symptoms were unresponsive to antibiotic therapy. Antibiotics used in Lyme disease and babesiosis kill *B burgdorferi* and *B microti* in the blood but cannot eliminate neurotoxins. Patients were amply and repeatedly treated with antibiotics for Lyme disease, with antibiotics and atovaquone for *Babesia*, and with toxin-binding protocols for antibiotic-resistant Lyme. Their delayed improvement in symptoms and VCS scores implies that *Babesia* also makes a neurotoxin that is removed much more slowly than are the putative Lyme neurotoxins. The “watershed” improvement evident after 9 weeks of atovaquone suggests that living *B microti* organisms, perhaps present in sanctuaries, were not eradicated by shorter courses of atovaquone with or without antibiotics in coinfecting patients. Further, the incremental objective improvement in VCS after the watershed permits the conclusion that eradication of *B microti* was followed by elimination of its neurotoxin.

This study had several limitations. Investigators were aware that symptoms or VCS scores may have improved to some extent regardless of treatment, even though self-healing had not occurred after a long time. Cholestyramine binds to and decreases the absorption of vancomycin, tetracycline, penicillin G, warfarin, and thiazide diuretics. To minimize this possibility, atovaquone and cholestyramine were given at separate times, and at least 1 hour elapsed between doses (as is recommended for cholestyramine and vancomycin). Nevertheless, some binding of atovaquone by cholestyramine was possible and may have attenuated the clinical response. An interaction study is needed to determine the optimal dosing times of these agents. Prolonged use of cholestyramine may reduce absorption of the fat-soluble vitamins A, D, E, and K. If the present study had been extended, careful monitoring for signs or symptoms of vitamin deficiencies would have been needed. Because repeat peripheral smears or cultures for *B microti* were not performed, investigators cannot confirm that clinical and VCS improvements are correlated with eradication of *B microti*.

The present study also left other questions unanswered: Does *B microti*, similar to *B burgdorferi*, release a toxin? Does upregulation of anti-inflammatory cytokines triggered by *B burgdorferi* toxins interfere with atovaquone’s lethal action on *B microti*? What is the role of proinflammatory cytokines in symptom induction and the course of coinfection? Is extravascular sequestration of viable *Babesia* organisms possible? What is the role of the extrachromosomal 35-kb plasmid DNA—homologous to the DNA of *Euglena*—in maintaining viable *Babesia* organisms despite antibiotic therapy?

Use of the atovaquone/cholestyramine regimen for Lyme disease/babesiosis coinfection would be expected to obviate the need for parenteral antibiotics, thus avoiding their inconvenience, safety risks, and expense. Patients in the study had been ill for so long that they were willing to undergo multiple courses of antibiotics, both oral and

parenteral. Currently, parenteral antibiotics are often prescribed for recurring or recalcitrant Lyme symptoms and babesiosis when oral antibiotics prove ineffective.

CONCLUSION

In view of the delayed clinical response observed in this study, longer-term evaluations of atovaquone/cholestyramine appear warranted. Efficacy and safety comparisons with currently recommended antibiotic regimens also may elucidate the ultimate place of atovaquone/cholestyramine in the treatment of patients with Lyme/babesiosis coinfection.

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REFERENCES

1. Bacon RM, Mead PS, Kool JL, Postema AS, Staples JE. Lyme disease—United States, 2001–2002. *MMWR Wkly*. 2004;53:365–369.
2. Centers for Disease Control and Prevention. Lyme disease. Epidemiology. Available at: <http://www.cdc.gov/ncidod/dvbid/lyme/epi.htm>. Accessed June 22, 2005.
3. Thompson C, Spielman A, Krause PJ. Coinfecting deer-associated zoonoses: Lyme disease, babesiosis, and ehrlichiosis. *Clin Infect Dis*. 2001;33:676–685.
4. Krause PJ, Telford S, Spielman A, et al. Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness. *JAMA*. 2006;295:1657–1660.
5. Shapiro ED, Gerber MA. Lyme disease. *Clin Infect Dis*. 2000;31:533–542.
6. Wormser GP, Nadelman RB, Dattwyler RJ, et al. Practice guidelines for the treatment of Lyme disease. *Clin Infect Dis*. 2000;31(suppl 1):S1–S14.
7. Bujak DJ, Weinstein A, Dornbush RL. Clinical and neurocognitive features of the post Lyme syndrome. *J Rheumatol*. 1996;23:1392–1397.
8. Krupp LB, Hyman LG, Grimson R, et al. Study and treatment of post Lyme disease (STOP-LD). *Neurology*. 2003;60:1923–1930.
9. Steiner I. Treating post Lyme disease. Trying to solve one equation with too many unknowns. *Neurology*. 2003;60:1888–1889.
10. Weinstein A, Britchkov M. Lyme arthritis and post-Lyme disease syndrome. *Curr Opin Rheumatol*. 2002;14:383–387.
11. Shoemaker R, House D. Use of pioglitazone to prevent intensification of persistent symptoms following cholestyramine treatment of patients with post-Lyme syndrome [abstract]. *Diabetes*. 2002;51:A133.
12. Cartwright MJ, Martin SE, Donta ST. A novel neurotoxin (Bbtox 1) *Borrelia burgdorferi*. Presented at: Meeting of the American Society for Microbiology; May 1999; Chicago, Ill.
13. Hudnell HK. Chronic Lyme disease may be caused by toxins. Available at: Lyme Times/www.lymedisease.org. Accessed June 25, 2005.
14. Kjemtrup AM, Conrad PA. Human babesiosis: an emerging tick-borne disease. *Int J Parasitol*. 2000;30:1323–1337.
15. Weiss LM. Babesiosis in humans: a treatment review. *Expert Opin Pharmacother*. 2002;3:1109–1115.
16. Krause PJ, Lepore T, Sikand VK, et al. Atovaquone and azithromycin for the treatment of babesiosis. *N Engl J Med*. 2000;343:1454–1458.

17. Gowda C. Natural antibodies to *Plasmodium falciparum* glycosylphosphatidylinositol (GPI) in an area with seasonal malaria transmission [abstract]. *Am J Trop Med Hyg.* 2002;67:154.
18. Johnson L, Shoemaker RC. Treatment of horses with equine protozoal meningoencephalitis (EPM) coinfecting with *Borrelia burgdorferi*: a case series. Presented at: Third Annual Meeting, International Lyme and Associated Diseases Society; October 3, 2002; Princeton, NJ.
19. Shoemaker RC. MMP9, visual contrast sensitivity, C3A, C4A, and HLA DR: new diagnostic aids in acute and chronic Lyme disease. Presented at: 54th Annual Meeting of the American Society for Tropical Medicine and Hygiene; December 11–15, 2005; Washington, DC. Abstract/presentation 695.
20. Hartmann F, Müller-Marienburg H. Indifekter Neurotoxinnacgweis durch den visual contrast sensitivity test bei Patienten mit einer chronischen Boreliose. *Med Welt.* 2003;54:248-251.
21. Hudnell HK, Shoemaker RC. Sick building syndrome: possible association with exposure to mycotoxins from indoor air fungi. Available at: <http://www.chronicneurotoxins.com/learn-more/latestAbstracts.cfm>. Accessed July 24, 2005.
22. Pavia CS. Current and novel therapies for Lyme disease. *Expert Opin Investig Drugs.* 2003;12: 1003-1016.
23. Schmidt P. New theory links neurotoxins with chronic fatigue syndrome, Lyme, MCS and other mystery illnesses. Available at: <http://www.immunesupport.com/library/print.cfm>. Accessed June 22, 2005.
24. Hartmann F, Müller-Marienburg H, Hopf-Seldel P. Über die cholestyramine der chronischen Boreliose. *Medizin.* 2004;1:14-19.
25. Shoemaker RC. *Markers for Chronic, Biotoxin-Associated Illness*. Madison, Wisc: American Association for Chronic Fatigue Syndrome; 2004.
26. Steere AC, Hutchinson GJ, Rahn DW, et al. Treatment of the early manifestations of Lyme disease. *Ann Intern Med.* 1986;99:22.
27. Questran® (cholestyramine) Powder [package insert]. Princeton, NJ: Bristol-Myers Squibb; June 1999.
28. Shoemaker RC, Hudnell HK. Possible estuary-associated syndrome: symptoms, vision, and treatment. *Environ Health Perspect.* 2001;109:539-545.
29. Shoemaker RC, House D. A time study of sick building syndrome: chronic, biotoxin-associated illness from exposure to water-damaged buildings. *Neurotox Teratol.* 2005;27:29-46.
30. Shoemaker RC, Rash JM, Simon EW. Sick building syndrome in water-damaged buildings: generalization of the chronic, biotoxin associated illness paradigm to indoor toxigenic fungi. In: Eckardt J, ed. *Bioaerosols, Fungi, Bacteria, Mycotoxins and Human Health*. Albany, NY: Eastern New York Occupational & Environmental Health Center; 2005:66-77.
31. Shoemaker RC, Hudnell HK, House D. A new approach to chronic ciguatera illness and successful treatment with cholestyramine. *Am J Trop Med Hyg.* 2000;62(suppl):160-161.
32. Shoemaker RC, Hudnell HK. Exposure to freshwater lakes with resident toxigenic cyanobacteria, *Cylindrospermopsis*, is associated with a chronic illness. Poster presented at: Centers for Disease Control and Prevention National Conference on *Pfiesteria*; November 18, 2000; Stone Mountain, Ga.
33. Kyne L, Farrel RJ, Kelly CP. *Clostridium difficile*. *Gastroenterol Clin North Am.* 2001;30:753.
34. Gronczewski CA. *Clostridium difficile* colitis. *Emedicine* 2003. Available at: www.emedicine.com/med/topic3412.htm. Accessed July 12, 2005.
35. Kurtz CB, Cannon EP, Brezzani A, et al. GT160-246, a toxin binding polymer for treatment of *Clostridium difficile* colitis. *Antimicrob Agents Chemother.* 2001;45:2340.
36. Hudnell HK, House D, Schmid J, et al. Human visual function in the North Carolina clinical study on possible estuary-associated syndrome. *J Toxicol Environ Health.* 2001;62:575-594.
37. Shoemaker RC. Residential and recreational acquisition of possible estuarine associated syndrome: a new approach to successful diagnosis and treatment. *Environ Health Perspect.* 2001;109:791-796.
38. Shoemaker RC, House D. Characterization of chronic human illness associated with exposure to cyanobacterial harmful algal blooms predominated by *Microcystis*. Presented at: International Symposium on Cyanobacteria and Harmful Algal Blooms; September 6–7, 2005; Research Triangle Park, NC.