

Detection of tick-borne infection in Morgellons disease patients by serological and molecular techniques

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Background: Morgellons disease (MD) is a skin condition associated with Lyme disease (LD) and tick-borne illness. Patients with this skin disorder experience ulcerative lesions that contain multicolored filamentous collagen and keratin inclusions. Infection with various species of *Borrelia* and other tick-borne pathogens has been detected in tissue and body fluid specimens from MD patients. We sought to explore this association further in a cohort of MD patients.

Patients and methods: Sera from 30 patients with MD were tested for antibody reactivity to antigens from the *Borrelia burgdorferi* (Bb) group and the relapsing fever *Borrelia* (RFB) group of spirochetes. Tissue and/or body fluid specimens from these patients were also tested for the presence of Bb and RFB infection using PCR technology. In addition, tissue and body fluid specimens were tested for the presence of *Bartonella henselae* using PCR, and formalin-fixed skin sections from a subset of patients were tested using fluorescent in situ hybridization (FISH) with *B. henselae*-specific DNA probes.

Results: Seroreactivity to Bb, RFB or both was detected in 63% of the cohort, while positive PCR testing for Bb, RFB or both was detected in 53% of the cohort. Overall, 90% of patients tested positive for exposure and/or infection with *Borrelia* spirochetes. *B. henselae* infection was detected by PCR in skin sections or body fluids from 20% of the subjects, and *B. henselae* FISH testing was positive in 30% of the dermatological specimens submitted for study.

Conclusion: The study demonstrates an association between MD and positive tests for both Bb and RFB spirochetes. In conjunction with previous studies, our study provides corroborative evidence linking MD to *Borrelia* infection and tick-borne illness.

Keywords: Lyme disease, *Borrelia burgdorferi*, Morgellons disease, relapsing fever *Borrelia*, *Bartonella*, tick-borne disease

Introduction

Morgellons disease (MD) is a dermatological condition characterized by spontaneously occurring lesions that contain filamentous inclusions and/or projections.^{1,2} The presence of unusual filaments is the key diagnostic feature of this dermopathy. These cutaneous filaments are firmly attached, lying under, embedded in, or projecting from skin, and can be white, black or vibrant hues of other colors.¹⁻⁴

Many physicians maintain that MD is synonymous with delusions of parasitosis (DOP), that it is purely psychogenic in origin and that the filaments are implanted textile fibers.^{5,6} In contrast, detailed scientific studies demonstrate that the disorder is associated with Lyme disease (LD) and other tick-borne illnesses and that the fibers are hair-like collagen and keratin filamentous growths produced by skin cells in the

deeper layers of the epidermis, the upper layers of the dermis and the root sheath of hair follicles.^{2-4,7,8} The coloration of blue fibers is due to melanin pigmentation, which is a finding that provides further proof that the fibers are not textile in origin.^{3,4,7,8}

MD is not exclusively a skin disorder. It can be accompanied by Lyme-like symptoms such as fatigue, joint inflammation, cardiac manifestations and neuropathy, which suggest a spirochetal relationship.^{1-4,9-12} Results from two independent cohort studies established that LD was present in almost all patients with this disorder. A 2010 study reported that 98% of its 122 subjects either tested serologically positive for LD or were clinically diagnosed with LD.¹ Similar results were reported in a 2018 study in which 100% of a cohort of 60 MD patients tested positive for LD.¹³ Two independent studies, one in Australian patients and one in North American patients, reported that MD occurs in a subset of approximately 6% of LD patients.^{13,14} This evidence suggests that MD may be a physiological reaction to the presence of *Borrelia* spirochetes and coinfecting pathogens in genetically predisposed LD patients.^{3,4}

Positive serology and/or molecular testing for *Borrelia burgdorferi* (Bb) and relapsing fever *Borrelia* (RFB) has been reported in MD patients, and positive tests for coinfecting tick-borne pathogens (*Babesia* spp., *Bartonella*, *Rickettsia*, *Ehrlichia* and *Anaplasma*) have also been described in patients with this dermatopathy.^{3,4,11-13,15} Both arthropod-vector (Bartonella spp.) and other non-arthropod-vector, human-associated pathogens (*Treponema denticola* and *Helicobacter pylori*) have been detected in skin specimens from MD patients.^{3,4} These pathogens may play an etiological role in MD evolution or may be exacerbating factors that worsen the condition.

Positive testing for *Borrelia* and other pathogens in MD patients has only recently been described, and therefore evidence of an infectious etiology is controversial. We sought to undertake a confirmatory study using both serological and PCR methods to demonstrate *Borrelia* infection in 30 randomly selected MD patients who met the key diagnostic criterion of MD. We also performed molecular testing for *Bartonella henselae* to ascertain the prevalence of this infection in MD patients.

Patients and methods

Patients and specimen collection

Adult participants from across North America either accessed the study via the Charles E. Holman Morgellons Disease Foundation or contacted the researchers independently.

Informed consent for specimen collection and study participation was obtained from each patient, and the identities of participants were kept strictly confidential. The study protocol was approved by the Western Institutional Review Board (WIRB), Puyallup, WA, USA. Additional written consent to publish data resulting from this study was obtained for each patient. The study was conducted in accordance with the Declaration of Helsinki.

Participants were included in the chronological order in which they volunteered. All volunteers were included providing they met the diagnostic criterion for MD: embedded or projecting white, black or colored cutaneous filaments documented by a healthcare professional. Prior testing for LD or other tick-borne illnesses was not required nor was that information provided to the researchers. Patient samples without demographic information were sent to the laboratory conducting the testing, and this information was only added to the data and analyzed after laboratory testing had been concluded. Participants who had lesions containing thickened callus material were invited to collect and submit skin specimens for further evaluation using histological sectioning and special staining for *Bartonella* spp.

Laboratory testing

All experimental tests were conducted at iGeneX Laboratory (Palo Alto, CA, USA), which is a high-complexity testing laboratory with Clinical Laboratory Improvement Amendments (CLIA) certification.

Borrelia Western blot (WB) testing

The WB assays were performed as described previously to detect IgM and IgG antibodies reactive to Bb and RFB.^{16,17} Nitrocellulose strips for the Bb WB were prepared from whole cell lysates of a mixture of the Bbss strains B31 and 297, as previously described.¹⁶ Nitrocellulose strips for the RFB WB were prepared from whole cell lysates of two RFB species, *Borrelia hermsii* and *Borrelia turcica*, that cross-react with RFB species known to infect human beings.¹⁷ Thus, the RFB WB assay used in this study detected antibodies reactive with RFB but did not differentiate this group at the species level.

The Bb and RFB strips were reacted with test sera from the MD patients, positive control sera from patients with confirmed LD and RFB and negative control sera from uninfected people. Each strip was labeled and soaked in 1 mL of diluent (100 mM Tris, 0.9% NaCl, 0.1% Tween 20% and 1% nonfat dry milk) for 5 minutes in a trough; then, 10 μ L aliquots of each test or control serum were added to

a separate strip in troughs. The strips were then incubated at ambient temperature for 1 hour, followed by three washes with wash buffer (KPL, Gaithersburg, MD, USA) at ambient temperature. The remaining final wash solution was aspirated, after which the strips were incubated for 1 hour with alkaline phosphate-conjugated goat antihuman IgG and IgM (KPL) to detect IgG and IgM, respectively, at 1:10,000 dilution for IgG and 1:6,000 dilution for IgM, then they were washed three times. The bands were then visualized by reaction with 5-bromo-4-chloro-3-indolylphosphatidyl-4-nitro-blue tetrazolium (BCIP/NBT; KPL). The enzymatic reactions were concluded by washing with distilled water at the point in time when a calibration control produced a visible band at 39 kDa. The calibration control consisted of an alkaline phosphatase-conjugated rabbit antibody to the 39/93 kDa Bb antigens (Strategic Biosciences, Stow, MA, USA) diluted in human serum, as previously described.¹⁶ Bands displaying a lower intensity than the calibration control were reported as negative.

Bb WB testing for IgM and IgG reactivity was interpreted as positive if two bands from the following 6 antigens were reactive: 23, 31, 34, 39, 41 and 93 kDa, with the following exceptions: for the IgG WB, if only the 31 and 41 or 31 and 93 kDa bands were reactive the assay was interpreted as indeterminate; and for the IgM WB, if only the 41 and 93 kDa bands were reactive the assay was interpreted as negative. Positive and negative controls were run with all Bb WB tests.

RFB WB testing for IgM and IgG reactivity was performed using separate strips for the *B. hermsii* and *B. turcica* species. RFB WB testing for either IgM or IgG was interpreted as positive if two bands from the following four antigens were reactive on the strips from either species: 21–23, Glycero-phosphodiester phosphodiesterase (GlpQ), 41 and 70–75kDa. Positive and negative controls were run with all RFB WB tests.

***Borrelia* PCR testing**

Clinical samples were tested by multiplex PCR for the detection of Bb, as described previously.¹⁸ PCR testing for RFB was performed using quantitative PCR (qPCR) targeting a 16S rDNA fragment. The *Borrelia* derivation of all positive Bb and RFB amplicons was confirmed by Sanger sequencing.

***B. henselae* PCR testing**

Clinical samples were tested by PCR amplification of a *Bartonella*-specific DNA target. The PCR primers were targeted to a 320 bp fragment at the 5' end of 16S rDNA. The *Bartonella* derivation of these 320 bp amplicons was confirmed by Sanger sequencing.

***B. henselae* fluorescent in situ hybridization (FISH) testing**

Formalin-fixed paraffin embedded skin tissue samples were deparaffinized as described previously.¹⁹ After the final wash with phosphate-buffered saline, the slides were dried and the skin sections were hybridized with a *Bartonella*-specific probe targeted to the 5' end of a *Bartonella* 16S rDNA fragment. The sections were labeled with Alexa 488 fluorescein isothiocyanate (FITC) dye in chaotropic buffer at 37°C for 30 minutes, followed by three 2-minute washes in wash buffer (saline–sodium citrate containing SDS) at ambient temperature. The sections were then counterstained with Sudan Black (Sigma-Aldrich Co., St Louis, MO, USA) for 20 minutes and dried in complete darkness. A drop of mounting medium was then added, and the sections were covered with a glass coverslip. Sections were read at 1,000× magnification using a light-emitting diode (LED) light source with custom filter sets (Fraen Corp, Cusago, Italy) for viewing green (excitation 490 nm; emission 529–530 nm band-pass filter) in an Olympus BX laboratory light microscope.

Results

Demographics

The patient cohort consisted of 3 male and 27 female subjects ranging in the age from 37 to 87 years with a mean age \pm SD of 58.1 ± 12.4 years. Patients resided in 10 US states (Texas, Virginia, California, Pennsylvania, Wisconsin, Indiana, Tennessee, Missouri, Washington and Colorado) and in three Canadian provinces (Alberta, Quebec and Ontario). The majority of patients resided in the province of Alberta (12) and the state of Texas (7). The geographic distribution and test results of patients are shown in Figure 1, and demographic data are listed in Table 1.

***Borrelia* WB testing**

Serum samples from all 30 patients with MD were tested for reactivity to Bb and RFB antigens by WB. Of the sera tested for each subject, 19/30 (63.3%) were interpreted as positive for either Bb and/or RFB. Of these, 4/30 (13.3%) reacted to Bb antigens alone, 11/30 (36.7%) reacted to RFB antigens alone, and dual reactivity with both Bb and RFB was detected in 4/30 (13.3%). Serum from 11/30 patients (36.7%) failed to react to antigens of either Bb or RFB group. *Borrelia* WB testing results are summarized in Table 2.

***Borrelia* PCR testing**

Blood specimens from all 30 patients and skin specimens from 10 patients with MD were submitted for DNA amplification

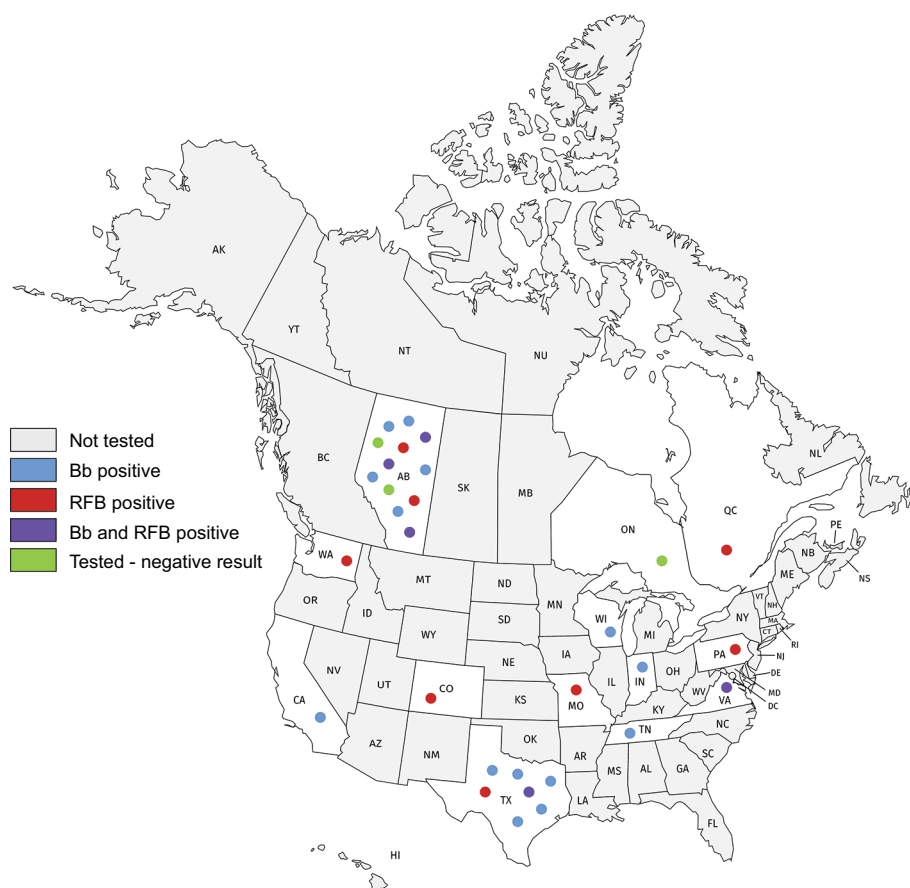


Figure 1 Geographic distribution of the MD patient cohort.

Abbreviations: Bb, *Borrelia burgdorferi*; MD, Morgellons disease; RFB, relapsing fever *Borrelia*.

using PCR technology. Amplicons to Bb DNA targets were obtained for specimens from 16/30 patients (53.3%). Amplicons to RFB DNA targets were obtained for specimens from 9/30 patients (30%). Of those patients from whom amplicons were obtained, 7/30 (23.3%) were positive for Bb amplicons but not RFB amplicons, 8/30 (26.7%) were positive for RFB amplicons but not Bb amplicons, 1/30 (3.3%) was positive for both Bb and RFB amplicons, and 14/30 (46.7%) subjects were negative. *Borrelia* PCR results are summarized in Table 3.

Combined *Borrelia* WB and PCR testing

All 30 patients with MD were tested for Bb and/or RFB infection by both WB and PCR amplification of target DNA. Of the subjects tested, 27/30 (90%) had a positive test result (either WB or PCR) for either Bb and/or RFB infection. Of these, 7/30 (23.3%) tested positive for Bb alone; 15/30 (50%) tested positive for RFB alone and 5/30 (16.7%) tested positive for both Bb and RFB. Only 3/30 (10%) tested negative for both spirochete species. Of the three patients whose tests were negative, two were taking antibiotics at the time

of sampling. In the MD cohort, 4/30 (13.3%) tested positive for Bb alone by both WB seroreactivity and PCR detection of Bb DNA, 4/30 (13.3%) tested positive for RFB alone by both WB seroreactivity and PCR detection of RFB DNA, and 0/30 (0%) tested positive for both Bb and RFB by WB seroreactivity to Bb and RFB antigens and PCR detection of Bb and RFB DNA. Combined testing of Bb and RFB by WB and PCR is summarized in Table 4.

B. henselae PCR testing

Blood specimens from all 30 patients and skin specimens from 10 patients with MD were submitted for PCR amplification of *B. henselae* PCR gene targets. Amplicons from blood and/or skin specimens were obtained for samples from 6/30 (20%) of the patients. *B. henselae* PCR results are summarized in Table 5.

B. henselae FISH testing

Formalin-fixed skin sections from a subset of 10 MD patients were stained with an FITC-labeled DNA probe

Table 1 Demographic data for the MD patient cohort

Patient ID no.	Age (years)	Sex	State or province
1	66	Female	TX
2	65	Female	AB
3	87	Female	TX
4	59	Female	AB
5	74	Male	AB
6	68	Female	TX
7	52	Female	TX
8	64	Female	TX
9	67	Female	IN
10	52	Female	VA
11	52	Female	QC
12	51	Female	WI
13	63	Female	AB
14	59	Female	MO
15	61	Female	AB
16	37	Female	TX
17	38	Female	AB
18	74	Female	CA
19	58	Female	PA
20	57	Female	AB
21	60	Female	TN
22	54	Female	ON
23	44	Female	AB
24	38	Female	AB
25	74	Male	AB
26	48	Female	TX
27	76	Female	AB
28	57	Female	AB
29	51	Male	CO
30	38	Female	WA
	Mean age \pm SD: 58.1 \pm 12.4 years	27 females, 3 males	

Abbreviation: MD, Morgellons disease.

for the detection of *B. henselae* target DNA. In the skin sections from these subjects, 3/10 (30%) demonstrated positive FISH tests. Of the three patients who tested positive by FISH, two were also positive by PCR amplification of target DNA. *B. henselae* FISH results are summarized in Table 5.

Discussion

Positive testing for *Borrelia* has consistently been detected in MD patients, and consequently a spirochetal etiology for this filamentous dermopathy has been proposed.^{3,4,11,12} Serological and/or molecular positivity for Bbss is most consistently detected in MD patients, but positive testing for the Bbsl species *Borrelia garinii* and the RFB species *Borrelia miyamotoi* and *B. hermsii* has also been found in patients with this dermopathy.^{3,4,11,12,15} Serological and/or molecular positivity for *Borrelia* species was detected in 90%

Table 2 WB test results for Bb and RFB

Patient ID no.	Bb WB	RFB WB	Infection detected
1	+ IgM	+ IgM	Bb/RFB
2	–	+ IgM	RFB
3	–	IND IgM	IND
4	+ IgM/IgG	–	Bb
5	–	–	–
6	–	+ IgM	RFB
7	–	–	–
8	IND IgG	+ IgM	RFB
9	–	+ IgM	RFB
10	–	–	–
11	–	–	–
12	–	+ IgM/IgG	RFB
13	–	+ IgM	RFB
14	+ IgM	–	Bb
15	+ IgM/IgG	+ IgM/IgG	Bb/RFB
16	IND IgM	+ IgM	RFB
17	+ IgM IgG	–	Bb
18	+ IgM	–	Bb
19	–	+ IgM/IgG	RFB
20	–	+ IgM	RFB
21	–	–	–
22	–	–	–
23	+ IgM	+ IgG	Bb/RFB
24	+ IgM/IgG IND	+ IgM/IgG	Bb/RFB
25	–	+ IgM	RFB
26	–	–	–
27	–	–	–
28	–	–	–
29	–	–	–
30	–	+ IgM	RFB
Total	8 + 2 IND	15 + 1 IND	4 Bb only 11 RFB only 4 dual Bb/RFB

Abbreviations: Bb, *Borrelia burgdorferi*; IND, indeterminate; RFB, relapsing fever *Borrelia*; WB, Western blot.

of the MD subjects in this study, supporting the association between *Borrelia* and MD. Although we did not find positive tests in all of our subjects, positive testing for Bb and RFB was present at a much higher rate than would be expected for the general population.^{20–22} Positive testing was more frequently detected for RFB than for Bb, and a significant number of patients had dual positivity for both Bb and RFB. Thus, the evidence demonstrates that MD is associated with a spectrum of *Borrelia* organisms rather than Bb alone. This variation may complicate the clinical picture in MD patients.

LD is an important disease in the northern hemisphere and is the most common vector-borne illness in the USA, justifiably drawing considerable worldwide attention.^{20,21} Relapsing fever is a neglected disease, even though it is a notable infectious vector-borne disease found in five out of

seven continents.^{22–24} Most RFB cases in the USA are caused by *B. miyamotoi*, *B. hermsii*, *B. lonestari*, *B. parkeri*, and *B. turicatae*, and occur predominantly in western states.^{25–28} Bb is transmitted by hardbodied (ixodid) ticks, while RFB are

primarily vectored by softbodied (argasid) ticks, and *B. recurrentis* is transmitted by the human body louse. Although most tickborne RFB species are transmitted by argasid ticks, *B. miyamotoi* and *B. lonestari* are transmitted by ixodid ticks.^{29,30}

Testing for *Borrelia* is primarily focused on LD. The commercial two-tier serological test for LD endorsed by the Centers for Disease Control and Prevention (CDC) is highly specific for Bbss because it is based on detection of a single Bbss laboratory strain, B31.^{16,31–34} False negatives are common and can be caused by factors such as patient seronegativity and genetic diversity among *Borrelia* strains.^{31–38} Thus commercial serological tests for LD fail to capture the genetic spectrum of *Borrelia* capable of causing infection.^{31–38} Serological testing for RFB is not commonly performed and is currently only available through a limited number of laboratories using “home brew” tests.^{17,29} Testing for MD and “Lyme-like illness” should embrace the diversity of *Borrelia*, including both Bb and RFB.^{17,33,36,38} WB band patterns for Bb and RFB are unique, and serological tests based on one group of antigens will not adequately detect the other.¹⁷ Our study emphasizes the necessity of testing for both Bb and RFB, not only in the MD population but in all suspected cases of tickborne disease.

PCR technology detected Bb and RFB infections in 53.3% of this cohort of MD patients, including cases that were missed by WB serological testing. For LD testing, the CDC does not recommend direct detection methods such as PCR detection of DNA, antigen detection, or culture.^{3,4,39} Serological techniques alone would miss valuable diagnostic evidence of infection for a sizable proportion of MD cases, particularly if the testing is only based on Bbss strain B31. Direct detection methods could offer useful clinical insight in suspected cases that are seronegative for LD.

Our study has several limitations, as outlined below. The cohort was too small to determine if there is a geographical pattern of infection among MD subjects. There were

Table 3 PCR test results for Bb and RFB

Patient ID no.	Bb PCR	RFB PCR	Infection detected
1	–	–	–
2	–	–	–
3	–	+ (Skin)	RFB
4	–	–	–
5	–	–	–
6	–	+	RFB
7	+	–	Bb
8	–	–	–
9	–	+	RFB
10	+	+	Bb/RFB
11	+	–	Bb
12	–	–	–
13	–	+ (Skin)	RFB
14	+	–	Bb
15	–	+	RFB
16	–	–	–
17	+	–	Bb
18	+	–	Bb
19	–	–	–
20	–	–	–
21	+	–	Bb
22	–	–	–
23	+	–	Bb
24	–	–	–
25	–	–	–
26	–	+ (Skin)	RFB
27	–	+ (Skin)	RFB
28	–	–	–
29	–	+ (Skin)	RFB
30	–	–	–
Total	8 +	9 +	7/30 Bb only 8/30 RFB only 1/30 dual Bb/RFB

Abbreviations: Bb, *Borrelia burgdorferi*; RFB, relapsing fever *Borrelia*.

Table 4 WB and PCR test results for Bb and RFB

Patients tested	WB	PCR	Combined WB and PCR	Either WB or PCR
Total Bb positive	8 (26.7%)	8 (26.7%)	4 (13.3%)	12 (40%)
Total Bb negative	22 (73.3%)	22 (73.3%)	26 (86.7%)	18 (60%)
Total RFB positive	15 (50%)	9 (30%)	4 (13.3%)	20 (66.7%)
Total RFB negative	15 (50%)	21 (70%)	26 (86.7%)	10 (33.3%)
Isolated Bb positive (RFB negative)	4 (13.3%)	7 (23.3%)	4 (13.3%)	7 (23.3%)
Isolated RFB positive (Bb negative)	11 (36.7%)	8 (26.7%)	4 (13.3%)	15 (50%)
Bb/RFB dual positive	4 (13.3%)	1 (3.3%)	0 (0%)	5 (16.7%)
Bb/RFB dual negative	11 (36.7%)	14 (46.7%)	22 (73.3%)	3 (10%)
Either Bb or RFB positive	19 (63.3%)	16 (53.3%)	8 (26.7%)	27 (90%)

Abbreviations: Bb, *Borrelia burgdorferi*; RFB, relapsing fever *Borrelia*; WB, Western blot.

Table 5 *B. henselae* PCR and FISH test results

Patient ID no.	PCR blood	PCR skin	FISH skin
1	–	ND	ND
2	–	ND	ND
3	–	+	–
4	–	ND	ND
5	–	ND	ND
6	+	–	–
7	–	+	+
8	–	–	–
9	–	ND	ND
10	–	ND	ND
11	–	–	–
12	–	ND	ND
13	–	–	–
14	–	ND	ND
15	–	–	–
16	–	ND	ND
17	–	ND	ND
18	–	ND	ND
19	–	ND	ND
20	–	ND	ND
21	–	ND	ND
22	–	ND	ND
23	–	ND	ND
24	–	ND	ND
25	–	ND	ND
26	–	+	+
27	–	+	–
28	–	ND	ND
29	–	+	+
30	–	ND	ND
Total	1+ blood	5+ PCR	3+ FISH

Abbreviations: *B. henselae*, *Bartonella henselae*; FISH, fluorescent in situ hybridization; ND, not done.

significant participant numbers only from the province of Alberta and the state of Texas (Figure 1 and Table 1). Our study selection was skewed to these two geographical areas because the Charles E. Holman Morgellons Disease Foundation is based in Texas and one of the authors of this study resides in Alberta. Thus referring doctors and volunteering participants had greater awareness of MD in these locations. While the numbers are not statistically significant because of the small cohort size and weighted recruitment, Bb was more frequently detected in Alberta subjects (5/10, 50%) than in Texas subjects (2/7, 28%). Further studies with larger numbers of patients using broader recruitment methodologies are needed to better understand the distribution pattern of different spirochetal infections.

Of the three subjects who tested negatively, two were taking antibiotics during the time of specimen collection. This may have influenced the test results for these two individu-

als, but no test for *Borrelia* is 100% sensitive.¹⁶ It is possible that the negative subjects were infected with *Borrelia* strains that were not captured by the techniques employed in this study. However, it is also possible that spirochetes other than *Borrelia* such as *Treponema* and *Leptospira* may elicit this dermatological condition, and there is published evidence supporting this hypothesis. Dual infection with the human periodontal spirochete *Treponema denticola* and Bb has been reported in MD subjects.^{3,4} Spirochetal infection with a variety of treponemal species causes a filamentous skin disorder in cattle, bovine digital dermatitis, that resembles MD.² In 1935 and 1938, cases of delusional parasitosis bearing symptomatic similarities to MD were published by the French physician Vié and the Swedish physician Ekbom who reported, respectively, that 6/8 and 3/7 of their case subjects had syphilis.⁴ Thus it is possible that in these cases the etiology of MD symptoms was a pathogen other than *Borrelia*.

Other pathogens have been detected in MD tissue and body fluid specimens, some of which can be tickborne (*Bartonella* and *Rickettsia* spp.) and others that are commonly encountered in humans (*Treponema denticola* and *Helicobacter pylori*).^{3,4,14,40–43} We detected *B. henselae* co-infection in our MD cohort using PCR (20% of blood and/or skin samples positive) and FISH (30% of skin samples positive). Thus positive testing for this pathogen appears to occur in a subgroup of MD patients, confirming the findings of previous studies.^{1,13} Co-infecting vector-borne pathogens such as *B. henselae* could be co-involved primary etiological factors in some MD patients or they may be secondary or exacerbating factors. Likewise, *T. denticola* and *H. pylori* could be primary or secondary etiological factors in MD evolution. As in previous studies,^{1,2,5,13} our cohort was comprised of primarily middle-aged Caucasian women. Etiological factors in MD such as pathogen variation, genetic background, hormonal influences, and immune status remain to be fully elucidated.^{3,4}

Conclusion

MD is associated with positive testing for spirochetal pathogens. Both Bb and RFB are encountered in the MD patient population and appear to be important primary etiological factors. MD may also be associated with tick-borne coinfections that could play a role in the evolution of dermopathy. The fact that RFB infection appears equal to Bb in terms of frequency highlights the complexity of *Borrelia* infection in this patient population and the need for better testing that reflects spirochetal genetic diversity. The role of *Borrelia* and coinfecting pathogens in the development of MD warrants further investigation.

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Author contributions

All the authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Disclosure

JSS is president and laboratory director of IGeneX Clinical Laboratory, Palo Alto, CA, USA. RBS is the owner of Union Square Medical Associates, a medical practice that treats tick-borne diseases in San Francisco, CA, USA. The other authors report no conflicts of interest in this work.

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