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Marianne J. Middelveen
International Lyme and Associated Diseases Society

Gheorghe M. Rotaru
Heartland Veterinary Clinic, Airdrie, Alberta, Canada

Jody L. McMurray
Heartland Veterinary Clinic, Airdrie, Alberta, Canada

Katherine R. Filush
University of New Haven

Eva Sapi
University of New Haven, ESapi@NewHaven.edu

See next page for additional authors

Authors

Marianne J. Middelveen, Gheorghe M. Rotaru, Jody L. McMurray, Katherine R. Filush, Eva Sapi, Jennie Burke, Agustin Franco, Lorenzo Malquori, Melissa C. McElroy, and Raphael B. Stricker

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Research Article

Canine Filamentous Dermatitis Associated with *Borrelia* Infection

Marianne J Middelveen[1], Gheorghe M Rotaru[2], Jody L McMurray[2], Katherine R Filush[3], Eva Sapi[3], Jennie Burke[4], Agustin Franco[4], Lorenzo Malquori[4], Melissa C McElroy[1] and Raphael B Stricker[1]*

Abstract

Background: Although canine clinical manifestations of Lyme disease vary widely, cutaneous manifestations are not well documented in dogs. In contrast, a variety of cutaneous manifestations are reported in human Lyme disease caused by the spirochete *Borrelia burgdorferi*. A recently recognized dermopathy associated with tickborne illness known as Morgellons disease is characterized by brightly-colored filamentous inclusions and projections detected in ulcerative lesions and under unbroken skin. Recent studies have demonstrated that the dermal filaments are collagen and keratin biofibers produced by epithelial cells in response to spirochetal infection. We now describe a similar filamentous dermatitis in canine Lyme disease.

Methods and Results: Nine dogs were found to have cutaneous ulcerative lesions containing embedded or projecting dermal filaments. Spirochetes characterized as *Borrelia* spp. were detected in skin tissue by culture, histology, immunohistochemistry, polymerase chain reaction (PCR) and gene sequencing performed at five independent laboratories. *Borrelia* DNA was detected either directly from skin specimens or from cultures inoculated with skin specimens taken from the nine canine study subjects. Amplicon sequences from two canine samples matched gene sequences for *Borrelia burgdorferi* sensu stricto. PCR amplification failed to detect spirochetes in dermatological specimens from four healthy asymptomatic dogs.

Conclusions: Our study provides evidence that a filamentous dermatitis analogous to Morgellons disease may be a manifestation of Lyme disease in domestic dogs.

Keywords

Morgellons disease; Lyme disease; *Borrelia burgdorferi*; Spirochetes; Dermopathy; Borrelial dermatitis; Dogs

Introduction

Dogs are the domestic animals most susceptible to Lyme disease (LD), and serve as both sentinels and as animal models to investigate tickborne illnesses [1-4]. Acute signs of LD caused by the spirochete *Borrelia burgdorferi* (Bb) – general malaise, fever, swelling of lymph nodes and pain – are non-specific, can disappear within a few days, and may be overlooked by the dog's owner, leading to chronic, disseminated LD in some dogs [2,5,6].

Weeks to months after infection–when Bb has disseminated into skin, joints and connective tissue–intermittent lameness associated with fatigue and moderate increase in body temperature is reported [2,5,6]. Neurological and cardiac manifestations are less frequent findings, while glomerulonephritis and renal tubular necrosis are described in some breeds–most commonly in Golden and Labrador Retrievers [7,8]. Cutaneous manifestations of Lyme disease in dogs are not well documented. Erythema migrans, the "bullseye" rash described in humans, is not reported in canine infections; however, dogs may develop a small reddish lesion approximately 1 cm in diameter around the tick bite, and this is thought to be an inflammatory response to the bite rather than to the Lyme spirochete [2,5,6].

Morgellons disease (MD) is a human dermopathy characterized by spontaneously-appearing, slowly-healing skin lesions containing filaments that are often extraordinarily colorful (blue, white, black, green, purple, pink and red) and lie under, are embedded in, or project from skin [9-17]. Many medical professionals consider MD to be a delusional disease and attribute the presence of colorful filaments to excoriation coupled with accidental or deliberate implantation of textile fibers [18,19]. In contrast, dermatopathology studies suggest that the filaments may lie under or project from unbroken skin, making it highly unlikely that they are self-implanted [9-17]. Furthermore, the unusual filaments are composed of keratin and collagen, and blue coloration in filaments is the result of melanin pigmentation, as shown by specific histological staining [14,15]. The unusual filaments can be visualized in skin under 50X–100X magnification and some filaments fluoresce under ultraviolet (UV) light, thus aiding the identification of MD cases [9-13].

Musculoskeletal and neurocognitive symptoms associated with Lyme disease and tickborne coinfections are frequently reported in this group of patients, and MD patients demonstrate seroreactivity to Bb antigens [12-14]. Histological, electron microscopic and PCR studies of dermatological tissue containing filamentous inclusions and/or projections derived from MD patients verified the presence of *Bb sensu stricto* (*Bbss*) and *Bb sensu lato* (*Bbsl*) spirochetes in MD clinical specimens [15-17]. Motile spirochetes characterized by molecular means as *Borrelia* spp were also cultured from MD skin, blood and vaginal specimens, indicating viability and systemic infection and suggesting that MD is a manifestation of LD [15,16]. These detailed molecular and histological studies confirm that MD is a true somatic infectious illness and not a delusional disorder [15-17].

We describe nine canine cases of *Borrelia* spirochetal infection associated with a wide variety of Lyme-like symptoms, including dermopathy with filamentous inclusions and/or projections. To our knowledge this is the first detailed description of canine clinical illness matching human MD.

Materials and Methods

Study subjects

Clinical histories for the dogs and their owners were obtained by two of the authors (GMR and JLM). Dermatological samples were

*Corresponding author: Raphael B. Stricker, M.D, International Lyme and Associated Diseases Society, Bethesda, Maryland, USA, Tel: +(415) 399-1035; Fax: (415) 399-1057; E-mail: rstricker@usmamed.com

collected at the Heartland Veterinary Clinic, Airdrie, AB, Canada. Written informed consent for participation in the study was obtained from all dog owners. Approval for sample testing was obtained from the Western Institutional Review Board, Puyallup, WA, and the Institutional Review Board of the University of New Haven, West Haven, CT.

Microscopic examination for dermal filaments

The presence of dermal filaments was confirmed in two ways: (1) either directly on the pet with a hand-held 50X microscope equipped with both a white LED light and a UV light, and/or (2) in dermal specimens removed from the animal and examined with a brightfield microscope at either 50X or higher magnification or with a microscope illuminated from above the specimen with white LED or UV light. Sectioned embedded or protruding filaments were also observed in some tissue sections with brightfield microscopy.

Cultures

Cultures were obtained using Barbour–Stoenner–Kelly H (BSK-H) complete medium containing 6% rabbit serum (Sigma Aldrich, #B8291) and the antibiotics phosphomycin (0.02 mg/l) (Sigma Aldrich), rifampicin (0.05 mg/l) (Sigma Aldrich), and amphotericin B (2.5 µg/l) (Sigma-Aldrich), as described previously [20]. Culture medium was inoculated with dermatological tissue removed from the dog by a scalpel blade and placed directly into the medium in the examination room. Cultures were incubated in a microaerophilic environment at 32°C, and culture fluid was examined by dark-field microscopy for spirochetes weekly for a total of 4 weeks of incubation. Cultures were processed for PCR by centrifuging the fluid at 10,000g for 25 minutes to concentrate spirochetes, retaining the pellet and discarding the supernatant. DNA was extracted using Qiagen DNeasy® Blood and Tissue Kits, prior to shipping. Culture fluid smears were fixed onto Superfrost plus® slides (Thermo Scientific) with acetone at -20°C for special staining.

Gömöri trichrome staining, dieterle staining and anti-Bb immunostaining

Dermatological specimens and/or culture smears were processed for histological and special staining at McClain Laboratories LLC, Smithtown, NY. Dermatological specimens were formalin-fixed, paraffin-blocked and sectioned for staining. Gömöri trichrome staining was performed as previously described [15]. Formalin-fixed dermatological sections and acetone-fixed culture slides were stained with silver nitrate using a standard Dieterle method, and anti-Bb immunostaining was performed as described previously [21]. Positive and negative controls of anti-Bb immunostains were prepared for comparison purposes using liver sections from uninfected mice and mice experimentally inoculated with Bb, as previously described [21]. Additional negative controls were: Gram-positive and Gram-negative commensal bacteria, normal human skin, human psoriasis skin, and human fungal-infected skin. For anti-Bb immunostaining, the following protocol was used: specimens on slides were reacted with unconjugated rabbit anti-Bb polyclonal antibody (Abcam ab20950) followed by incubation with an alkaline phosphatase probe (Biocare Medical #UP536L) then by a chromogen substrate (Biocare Medical #FR805CHC) and counterstained with hematoxylin. Optimal antibody dilutions were determined by titration. In addition, Dieterle staining and anti-Bb immunostaining was performed on a scab from control dog CC-1.

Molecular testing

PCR - University of New Haven, West Haven, CT: DNA from tissue was obtained by overnight lysis in 180 µl tissue lysis buffer (Qiagen) and 20 µl Proteinase K (Qiagen) at 56°C in a shaking water bath, followed by phenol/chloroform extraction the next day. Extracted DNA was suspended in 50-100 µl 1X TE buffer.

A TaqMan assay targeting a 139-bp fragment of the gene encoding the *Borrelia* 16S rRNA was used for the real-time detection of *Borrelia* DNA [22]. Final volumes of 20 µl with 900 nM of each primer, 200 nM of probe, and 10 µl of 2X TaqMan Universal PCR Master Mix (Applied Biosystems) were used and amplifications were conducted on a CFX96 Real-Time System (Bio-Rad) with cycling conditions of 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Fluorescent signals were recorded using CFX96 Real-Time software and Cq threshold automatically set. Reactions were performed in triplicate concurrently with positive (*Bb sensu stricto* strain B-31) and negative (water) controls. Reactions with additional negative controls (water, normal human foreskin, normal skin from two Lyme patients, and normal skin from one Morgellons patient) were performed to assure the accuracy and specificity of the assays. *Borrelia* DNA was not detected in any of the negative controls.

Nested PCR with primers for the 16S rRNA and pyrG genes was performed as previously described [22-25]. "Outer" primers were used in the first reaction and "inner" primers were used for the nested reaction, with 1 µl of PCR product from the first reaction used as template for the second [25]. Cycling parameters were as follows: 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C for 1 minute, annealing for 1 minute (temperature based on the primer set used), and extension at 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. PCR products were visualized on 1-2% agarose gels. For samples with amplicons that had DNA of suitable quality, Sanger sequencing was performed.

PCR - Australian Biologics, Sydney, Australia: Bb detection by real-time PCR targeting the 16S rRNA gene and endpoint PCR targeting the rpoC gene was performed as previously described [17,26] using the Eco™ Real-Time PCR system with software version 3.0.16.0. DNA was extracted from tissue using the QIAamp 96 DNA Mini QIAcube HT (Qiagen). Runs were performed in duplicate with positive (Vircell Amplirun *Borrelia* DNA) and negative controls using primers for the *Borrelia* 16S rRNA gene target [17]. Thermal profiles for analyses were performed as follows: polymerase activation for 10 minutes at 95°C then PCR cycling for 40 cycles of 10 seconds at 95°C dropping to 60°C sustained for 45 seconds. The magnitude of the PCR signal generated (ΔR) for each sample was interpreted as positive or negative when compared to positive and negative controls. Eluted water was also included with the negative controls to confirm non-contamination of extraction. Extraction control (Bioline BIO-3532) was used as an internal control and to confirm the efficiency of the extraction. Threshold levels are automatically set by the Eco™ system. Negative control runs showed no change in the magnitude of the PCR signals, indicating the absence of detectable *Borrelia* DNA. For samples with amplicons that had DNA of suitable quality, Sanger sequencing was performed.

PCR - Mount Allison University, New Brunswick, Canada: Tissue samples were placed in a microcentrifuge tube with 200 µl of AquaGenomic solution (Multitarget Pharmaceuticals). The tissue was homogenized with a micropestle and digested with 0.5 µl (10

μg) of proteinase K, and the samples were incubated at 60°C for 2 hours, then 95°C for 10 minutes. The digested sample was vortexed and centrifuged at 12,000g for 5 minutes. The supernatant (~180 μl) was removed and the DNA precipitated with 0.8vol of isopropanol. Following mixing, the samples were centrifuged at 12,000g for 5 minutes and the supernatant discarded. The tubes were rinsed twice with 70% ethanol, the pellet dried and DNA resuspended in 100 μl of 1mM Tris-HCl (pH 7.6) and stored at -20°C.

Nested PCR was used to test for *B. burgdorferi* OspC and membrane protein BB 0006 (AAC66397.2/ WP_002658381.1).

Primers were as follows:

Primer	Forward	Reverse
OspC Outside	GTA ATA ATT CAG GGA AAG ATGG	CAG CAC CTT TAG TTT TAG TAC C
OspC Inside	CTA ATG CGG TTT TAC TTG CTG	GTT TTT AAA ATG GCT TCT TTT GC
BB 0006 Outside	GCT CAA GAA TAT TTC CAA TCA AC	GTC GAT ATA AAG TTG ATA GTT CTG
BB 0006 Inside	CAG CTA ATA TGG ATC CAA TGC	GCT GTT GTA TTT TTT GCT TTC AG

For the first round, each reaction contained 12.5 μl of GoTaq® Green Master Mix (Promega), 8.5 μl of nuclease free water, 1 μl of each outside primer and 2 μl of extracted DNA. Reactions for the second round were set up similarly but used inside primers and 2 μl of product from the first round as the input DNA. The program for the nested PCR reaction was an initial denaturing at 95°C for 5 minutes followed by denaturing at 95°C for 15 seconds, annealing at 55°C (outside primers) or 58°C (inside primers) for 60 seconds and extension at 72°C for 45 seconds, repeated for 35 cycles, with a final extension at 72°C for 5 minutes. All PCR reactions were run with multiple negative controls to detect aerosol or solution contamination.

PCR products were visualized on a 1.2% (w/v) SB-agarose gel containing 5 μl of 10,000 X SYBR Safe stain (Edvotek #608). A GeneDireX 100bp DNA ladder was run alongside the PCR products to allow for size determination (GeneDireX, DM001-R500). Gels were imaged using a Fluor-S MultiImager or a DyNA Light Dual Intensity UV Transilluminator. Nested PCR products of appropriate size and quantity were sent to Nanuq (McGill University and Genome Quebec Innovation Center) for sequencing. Resulting chromatograms were analyzed using 4 Peaks. Consensus sequences were determined and imported into SeaView [27] for alignment. DNA extraction, PCR reactions and gel electrophoresis were all performed in different rooms. To control for amplicon contamination, the primers were designed for this experiment and had not been previously used.

Results

Clinical histories

Samples were collected from nine dogs with dermatological lesions and four randomly selected control dogs without dermatological pathology. The control dogs (two males and two females) were a West Highland White Terrier, a Whippet-Border Collie cross, a Beagle cross and a Golden Retriever. None of the control dogs had evidence of LD. Clinical histories for the nine affected dogs are summarized in Table 1. The pet owners' familiarity with MD is summarized in Table 2. Brief clinical summaries are outlined below.

Case C-1: In 2013, a 6-year-old male neutered English Bulldog presented at Heartland Veterinary Clinic. The dog had a history of tick exposure in Alberta, Canada. Clinical symptoms began in December 2012 and included spontaneously-appearing ulcerative skin lesions, weight gain, personality change (from friendly to aggressive), and later (mid July 2013) the development of a severe urinary tract infection. Painful, pruritic lesions first developed on the head and between toes, then spread to his back (Figure 1A).

In February 2013, the dog was referred to a veterinarian specialist in dermatology and was diagnosed with atopic dermatitis. *Staphylococcus pseudointermedius* was identified from a skin culture and allergy testing indicated an allergy to mugwart. Various treatments including topical antibiotics, antiseptic solutions, antifungal agents, steroids and topical cyclosporine resulted in little benefit.

In August 2014, multicolored fibers were noted to be projecting from the skin lesions, and the similarity of this condition to MD as described in humans was recognized. The dog's owners were not familiar with either LD or MD and had not noticed the microscopic dermal filaments. Gross microscopic examination revealed both embedded and protruding multicolored dermal filaments (white, pink, bluish purple and teal) (Figure 1B). These fibers fluoresced under UV light. Samples of skin were taken for histological studies and *Borrelia* culture, and serological testing was negative. However, confirmation of spirochetal infection resulted from the techniques described in this study.

The dog was then treated with antibiotic therapy targeting LD. He was first treated with doxycycline, but an allergy to this antibiotic resulted in a switch to cephalexin. The condition responded very well. A relapse occurred when antibiotic therapy was halted and necessitated resumption of therapy, this time with metronidazole and azithromycin. Other treatments included a short course of prednisone for swelling in the interdigital glands, and topical cyclosporine with

Table 1: Canine subject data. IFA, immunofluorescence assay. SNAP 4DX®, enzyme linked immunosorbent assay (ELISA) for heartworm disease, ehrlichiosis, Lyme disease and anaplasmosis.

ID#	Breed	Age, years	Sex (Neutered, Spayed)	Serology	Residence
C-1	English Bulldog	6	Male (N)	IFA and SNAP 4Dx® negative (IDEXX)	Alberta, Canada
C-2	English Bulldog	7.8	Female (S)	Not performed	Alberta, Canada
C-3	Golden Retriever	5	Female (S)	SNAP 4Dx® negative (IDEXX)	Colorado, USA
C-4	Miniature Schnauzer	7.8	Female (S)	Not performed	Alberta, Canada
C-5	Bullie	3	Male	Not performed	Texas, USA
C-6	Chihuahua	6.5	Male (N)	SNAP 4Dx® negative (IDEXX)	Kentucky, USA
C-7	Miniature Schnauzer	6	Female (S)	Not performed	Alberta, Canada
C-8	English Bulldog	5.8	Female (S)	Not performed	Alberta, Canada
C-9	Beagle Pug	0.6	Female (N)	Not performed	Alberta, Canada

Table 2: Pet owner familiarity with Morgellons disease (MD).

ID #	Owner had lesions diagnosed as MD	Owner had prior knowledge of MD
C-1	No	No
C-2	No	No
C-3	Yes	Yes
C-4	No	No
C-5	Yes	Yes
C-6	Yes	Yes
C-7	No	No
C-8	No	No
C-9	No	No

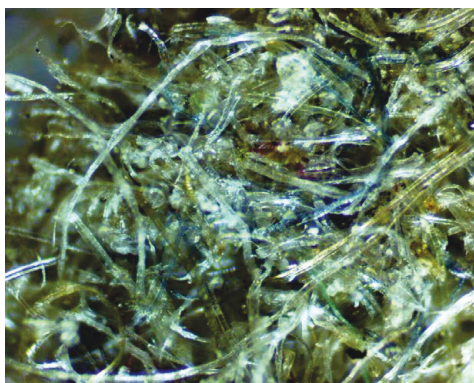**Figure 1A:** Painful pruritic lesions on back of dog C-1.

Figure 1B: Gross microscopic view of multicolored dermal filaments from dog C-1.

ketoconazole for management of pruritic symptoms. The skin lesions resolved over several months (Figure 1C), and the dog's personality reverted back to friendliness.

Case C-2: In December 2014, a spayed female 7.8-year-old English Bulldog (same owners as dog C-1), presented at Heartland Veterinary Clinic with lesions similar to those seen in dog C-1. The dog had a history of tick exposure in Alberta, Canada, similar to

dog C-1. Because of the previous experience, filamentous borrelial dermatitis was suspected. Examination with a dermatoscope revealed the presence of white, pink, bluish-purple, and teal fibers protruding from unbroken skin. These fibers fluoresced under UV light. Examination of dermatological specimens revealed pink, blue and white fibers. Based on the experience with dog C-1, dog C-2 was promptly treated with doxycycline and cephalexin, and her skin lesions resolved completely.

Case C-3: In 2005, a 5-year-old spayed female Golden Retriever presented at an undisclosed veterinary clinic located in Colorado, USA, with pruritic nodular and ulcerative lesions. The dog had a history of tick exposure in Colorado. She was diagnosed with allergic dermatitis concurrent with secondary staphylococcal infection, and she had been treated for her condition for the past 4 years by her current veterinarian. Symptoms had waxed and waned during that time period, generally worsening during summer months. Ongoing treatment included prednisone and antibiotic therapy given primarily during summer months with doxycycline, metronidazole, cefpodoxime and a topical spray containing gentamicin and betamethasone. There was significant improvement with this treatment. The dog's owner had a history of MD and reported to her veterinarian that the dog was similarly afflicted. She requested that the attending veterinarian submit specimens for this study.

The attending veterinarian was amenable to further investigation, took two punch biopsies from afflicted areas of skin and collected dermatological detritus from the dog's lesions that were then submitted for study. Serological evidence of LD by the SNAP 4Dx® test (IDEXX Laboratories, Westbrook, ME) was negative. Dermatological specimens revealed projecting and embedded multi-colored (white and blue) filaments. As a result of this study, treatment with oral antibiotics was restarted. To date, this therapy has helped though not eliminated the condition.

Case C-4: A 7.8-year-old spayed female Miniature Schnauzer presented at Heartland Veterinary Clinic. The dog had a history of tick exposure in Alberta, Canada. Symptoms included hair loss accompanied by crusty, pruritic and painful lesions, on the back and

Figure 1C: Back of dog C-1 following antibiotic treatment showing resolution of skin lesions.

sides. Under magnification with a 50X hand-held microscope, white, pink, bluish-purple and teal fibers that fluoresced under UV light were seen in the skin lesions. As a result of this study, she was treated with a cephalosporin antibiotic for four months, and the lesions resolved. The dog subsequently left the care of Heartland Veterinary Clinic due to relocation of her owners to a different city, and her long-term clinical outcome is unknown. The owners had very limited knowledge of LD and no prior knowledge of MD.

Case C-5: A 3-year-old male Bullie (Pit Bull/Staffordshire Terrier cross) presented at a veterinary clinic in Spring, Texas, USA in early 2015. The dog had a history of tick exposure in Texas. Primary symptoms included welts and ulcerative skin lesions on his back associated with hair loss, and intense pruritus severe enough that it caused him to dig at his skin. Symptoms started when he was about one-year-old. The attending veterinarian considered allergies and skin infection in the differential diagnosis. However, the dog's owner suffers from MD symptoms and after explaining the similarities between his condition and his dog's, the veterinarian was amenable to including the dog in this study. Gross microscopic examination revealed dermal filaments. The dog was treated with doxycycline and metronidazole, plus prednisone to alleviate symptoms. This treatment was effective in reducing, but not completely eliminating, the dermopathy.

Case C-6: For several years, a 6.5-year-old neutered male Chihuahua had symptoms that started when the dog and his owner resided in Kentucky, USA, where the dog had been exposed to ticks. Symptoms included dermopathy that began with the development of black specks, discolored skin, and swelling around his anus and penis. The owner reported changes to the dog's hair with the texture becoming coarse, hair loss where lesions were present, and the projection of unusual filaments from afflicted areas of skin. Other symptoms included depression, gastrointestinal problems, and the lack of an anal sphincter reflex. The presence of lesions, skin discoloration, and neuropathy were noted and confirmed by a veterinarian in Kentucky.

The dog was sent to various specialists including a neurologist. Various topical treatments failed, and tests including a negative SNAP 4Dx® revealed no useful clinical information. Microscopic examination of a dermatological specimen revealed embedded filaments, predominantly white. Although spirochetal infection was detected during the course of this study, the dog and owner moved to another city and have not yet found a veterinarian who is willing to try antibiotic therapy. The owner also has symptoms of MD and is familiar with the condition.

Case C-7: A 6-year-old salt-and-pepper spayed female Miniature Schnauzer presented at Heartland Veterinary Clinic in July 2015. The dog had a history of tick exposure in Alberta, Canada. She was spayed in August 2014 and vaccinated in September 2014 by her regular veterinarian. During that period of time her skin was reported to be normal. She was referred by her regular veterinarian to Heartland Veterinary Clinic in July 2015 after she developed numerous small, pruritic sores on her back. She was treated with prednisone and amoxicillin without benefit. By August 2015 the skin lesions had worsened and examination with a dermatoscope revealed white, pink, bluish purple and teal fibers that fluoresced under UV light. Her treatment was changed to doxycycline and a topical triamcinolone-neomycin-nystatin-gramicidin cream, and after two weeks on this regimen her skin returned to normal. An update by her owner in

January 2016 revealed that her skin had remained free of lesions until very recently. She currently has only one small lesion, and antibiotic therapy will be resumed.

Case C-8: In July 2015 a 5.8-year-old spayed female English Bulldog presented at Heartland Veterinary Clinic with dermatitis and an ear infection. The dog had a history of tick exposure in Alberta, Canada. She was treated with oral trimeprazine tartrate and prednisolone for the dermatitis and started treatment with oral cephalexin and marbofloxacin-clotrimazole-dexamethasone eardrops for the ear infection. Testing for thyroid function was normal.

She returned in early January 2016 with worsening dermatitis, patchy hair loss and pruritus. Examination with a 50X hand-held microscope revealed pink and blue embedded and projecting dermal filaments. She was not tested for Lyme disease. Treatment with doxycycline was started. On her follow-up visit in late January 2016, she showed marked improvement and her skin lesions had healed. There were a few blue dermal filaments that were visible under UV fluorescence.

She continued to take doxycycline for three months. Although the skin lesions cleared, pruritus persisted. The antibiotic was discontinued, and she has continued oral trimeprazine tartrate and prednisolone without recurrence of the lesions.

Case C-9: A 5-month-old spayed female beagle/pug cross was seen at Heartland Veterinary Clinic with worsening alopecia. The dog had a history of tick exposure in Alberta, Canada. She was diagnosed with *Demodex* infestation in September 2015, and she was treated for pruritus and mild alopecia with topical imidacloprid and moxidectin. Her skin symptoms resolved by December 2015, but alopecia recurred in March 2016. She was negative for mites, but examination with a 50X hand-held microscope revealed pink and blue dermal projecting and embedded dermal filaments that fluoresced under ultraviolet light. Based on this finding, the dog was treated with doxycycline and her skin lesions resolved over three months.

Evidence of dermal filaments

Dermatological findings in the nine dogs are summarized in Table 3. Dogs C-1, C-2, C-4, C-7, C-8 and C-9 were examined with a 50X hand-held microscope for in situ verification of dermal filaments. Unusual multicolored filaments (white, pink, bluish-purple and teal) projecting from skin lesions under LED white light were observed in these dogs. Unusual filaments projecting from skin lesions that fluoresced brightly under UV illumination were observed in dogs C-1, C-2, C-4, C-7, C-8 and C-9.

Using 100X magnification, gross dermatological specimens from dogs C-1 to C-9 demonstrated embedded and projecting filaments approximately 10-40 μm in diameter. Embedded multicolored filaments (white, pink, blue and teal) were observed in dermatological scrapings from dogs C-1, C-8 and C-9. Blue and white filaments were observed in dermatological scrapings from dogs C-2 and C-3, and white filaments were detected in dogs C-4 to C-7. Dermatological specimens collected from all subjects demonstrated embedded, white, unusual filaments at 100X magnification that fluoresced under UV light.

Dermatological specimens from dogs C-3 and C-4 had additional coarse black dermal filaments that were thicker than normal canine hair. The black filaments from dog C-3 had been firmly attached to the skin, and were plucked out by the attending veterinarian with

Table 3: Unusual dermatological findings and filament verification.

| ID	In situ 50X magnification	Gross examination 100X	100X examination of sectioned embedded filaments
C-1	White, pink, bluish-purple and teal projecting fibers that fluoresced under UV light	Embedded blue, red and white filaments 10 to 40 μm in diameter	Filamentous inclusions with hollow medulla and solid cortex; Gömöri trichrome determined keratin and collagen composition
C-2	White, pink, bluish-purple and teal projecting fibers that fluoresced under UV light	Attached blue and white filaments 10 to 40 μm in diameter	
C-3		Embedded blue, and white filaments 10 to 40 μm in diameter; coarse black hairs with visible scaling and deformed follicular bulbs; thickened hyperkeratotic follicular casts with blue filamentous projections	
C-4	White, pink, bluish-purple and teal projecting fibers that fluoresced under UV light	Embedded white filamentous fibers 10 to 40 μm in diameter; deformed hair follicles	
C-5		Embedded white filamentous inclusions 10 to 40 μm in diameter	Filamentous inclusions 10 to 40 μm in diameter with hollow medulla and solid cortex; one fiber nucleated shaft towards bulb, losing nuclei farther away from point of origin
C-6		Embedded white filaments 10 to 40 μm in diameter	Filamentous inclusions 10 to 40 μm in diameter with hollow medulla and solid cortex
C-7	White, pink, bluish-purple and teal projecting fibers that fluoresced under UV light	Embedded white filaments 10 to 40 μm in diameter	Filamentous inclusions 10 to 40 μm in diameter with hollow medulla and solid cortex
C-8	White, pink, bluish-purple and teal projecting fibers that fluoresced under UV light		
C-9	Pink and blue projecting fibers that fluoresced under UV light		
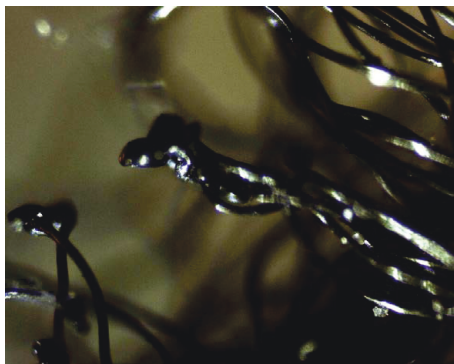
Figure 2: Dermatological specimen from dog C-3 showing coarse black dermal filaments with multiple hairs originating from a single bulb, and/or multiple bulbs fused together. 1000X.

difficulty and then submitted for study. Microscopic examination at 1000X revealed that the black fibers were coarse and had scaling consistent with hairs. The follicular bulbs revealed deformities with multiple hairs originating from a single bulb, and/or multiple bulbs fused together (Figure 2).

Dog C-1 had large areas of dermopathy, and sections prepared for histological studies contained filamentous inclusions suitable for performing Gömöri trichrome staining to confirm the collagen and keratin composition of filaments. The dermatological specimens revealed embedded filaments that consisted of a hollow medulla surrounded by a solid cortex, morphologically consistent with the filaments seen in human MD tissue. Some sectioned filaments stained green, indicating collagen composition (Figure 3), and some stained irregularly with both green and red indicating both keratin and collagen content.

A dermatological specimen from dog C-5 stained with anti-Bb immunostain revealed a fiber with a nucleated shaft (Figure 4). The nuclei stained blue and were more concentrated towards the point of origin of the fiber. A coiled red-staining spirochete was attached to the fiber shaft (Figure 4).

Microscopic examination of dermatological sections for evidence of infection

Microscopic findings for evidence of infection in dermatological samples from the nine dogs are summarized in Table 4.

Dieterle staining: Stained slides were examined at 400X and 1000X magnification. Silver nitrate staining revealed slender bacteria or spherules morphologically consistent with *Borrelia* spirochetes, as well as granular forms (spherules) in histological sections of dermatological tissue obtained from dogs C-1 to C-3 and C-5 to C-7. Staining in tissue sections often showed jagged spirochetes, as seen in the section from dog C-7 (Figure 5A). Dieterle staining of a scab from control dog CC-1 was negative (Figure 5B).

Anti-Bb immunostaining: Stained slides were examined at 400X and 1000X magnification. Anti-Bb immunostaining demonstrated cherry-red, strongly-positive staining in histological sections of dermatological tissue obtained from C1, C-2, C-3, C-5 and C-6. Staining was weaker though positive on sections from dog C-4. In sections from dogs C-1 and C-3, well-defined spirochetes were clearly visible, particularly in sections from C-1 (Figure 5C). Positive extracellular staining was present in tissue, probably due to secreted *Borrelia* antigens and/or lysis of *Borrelia* spirochetes. Anti-Bb immunostaining of a scab from control dog CC-1 was negative (Figure 5D).

Microscopic examination of culture

Darkfield examination of fluid: BSK-H fluid was inoculated with dermatological tissue from dogs C-1, C-2, C-4, C-7, C-8 and C-9. After incubation for four weeks, the cultures were examined with darkfield microscopy at 400X and/or 1000X magnification, revealing predominantly spherules approximately 0.1 to 1 μm diameter and occasional motile spirochetes, some with visible helical morphology, approximately 0.5 μm diameter and 10-15 μm long in the culture fluid, as seen in the sample from dog C-1 (Figure 6A).
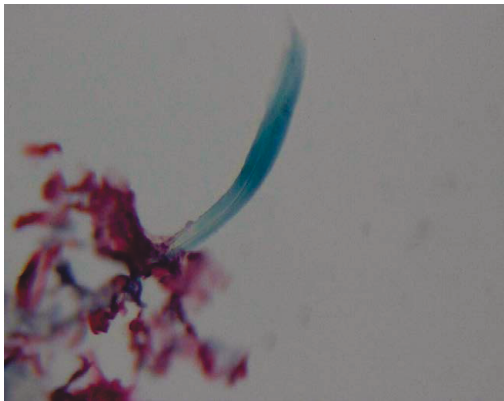
Figure 3: Dermatological specimen from dog C-1 showing filaments that stained green, indicating collagen composition. Gömöri trichrome stain, 1000X.
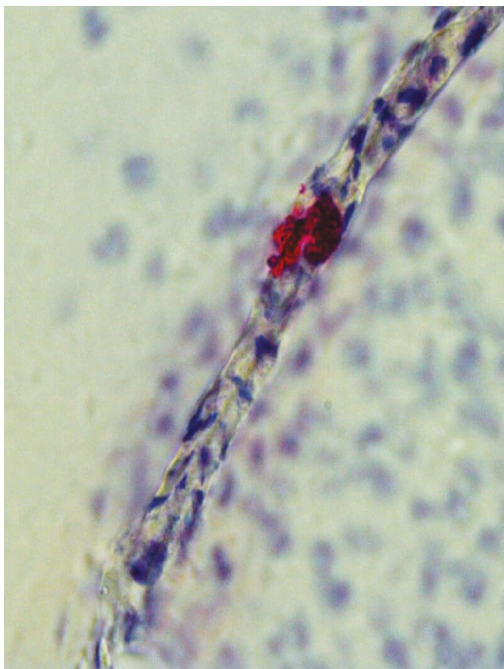

Figure 4: Dermatological specimen from dog C-5 showing a fiber with a nucleated shaft and attached red-staining spirochete. Anti-*Borrelia* immunostain, 1000X.

Dieterle staining: Stained culture smears for samples from C-1, C-2, C-4 and C-7 were examined at 400X and 1000X magnification. Silver nitrate staining revealed spherules and longer bacteria morphologically consistent with *Borrelia* spp., as seen in the sample from dog C-4 (Figure 6B).

Anti-Bb immunostaining: Stained culture smears for C-1, C-2, C-4 and C-7 were examined at 400X and 1000X magnification. Anti-Bb immunostaining demonstrated cherry-red, strongly-positive staining with spherules and longer bacteria morphologically consistent with *Borrelia* spp., as seen in the sample from dog C-4 (Figure 6C).

Molecular testing

Dermatological specimens from the nine affected dogs and four control dogs were submitted for PCR detection of *Borrelia* DNA at three independent laboratories: Mt Allison University, Sackville, NB; University of New Haven, West Haven, CT; and Australian Biologics, Sydney, Australia. These samples included dermatological tissue and/or DNA extracted from skin culture pellets. The positive results for DNA amplification of *Borrelia* DNA targets are summarized in Table 5. All samples were run with positive and negative controls. *Borrelia* DNA was not detected in any of the control dogs at the three independent laboratories (Table 6).

Amplicon sequences from dermatological tissue were obtained from dogs C-3 and C-7, and BLAST analysis of the forward and reverse sequences are shown in Figure 7. The amplicon sequence from dog C-3 demonstrated 99% homology with the *B. burgdorferi sensu stricto* strain B31 pyrG gene (Figure 7A and 7B). The amplicon sequence from dog C-7 demonstrated 99% homology with the *B. burgdorferi sensu stricto* strain B31 16S gene (Figure 7C and 7D).

Discussion

In this study, we describe nine dogs presenting at veterinary

Figure 5A: Dieterle silver staining of dermatological specimen from dog C-7 showing spirochete morphologically consistent with *Borrelia*. 1000X.
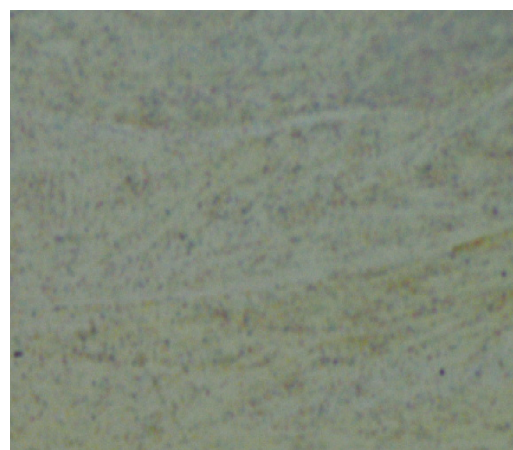

Figure 5B: Dieterle silver staining of dermatological specimen from control dog CC-1 showing absence of spirochetes. 1000X.
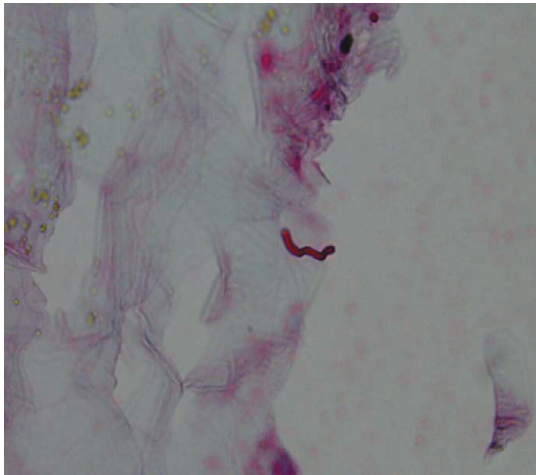
Figure 5C: Anti-*Borrelia* immunostaining of dermatological specimen from dog C-1 showing cherry-red stained spirochete. 1000X.
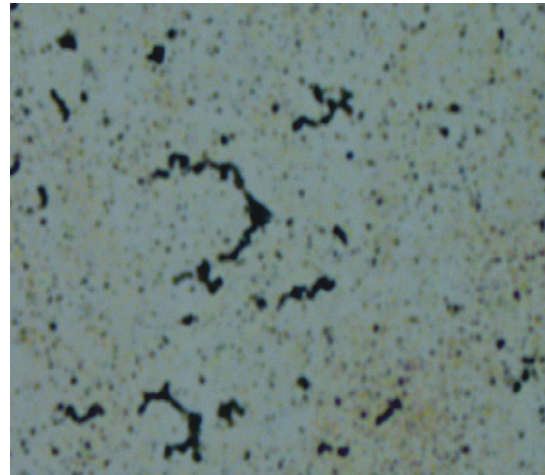

Figure 6B: Dieterle silver staining of dermatological culture fluid from dog C-4 showing spirochetes morphologically consistent with *Borrelia*. 1000X
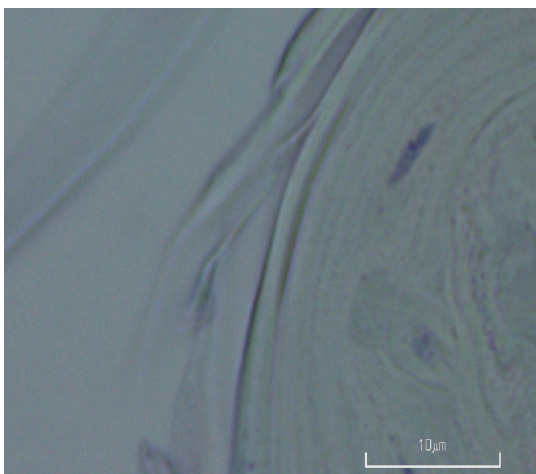
Figure 5D: Anti-*Borrelia* immunostaining of dermatological specimen from control dog CC-1 showing absence of spirochetes. 1000X.
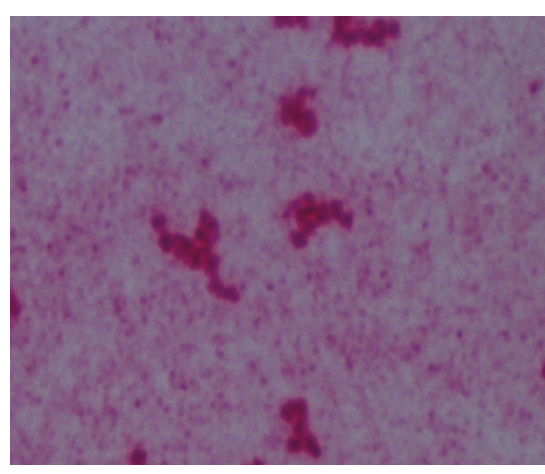
Figure 6C: Anti-*Borrelia* immunostaining of dermatological culture fluid from dog C-4 showing spirochetes morphologically consistent with *Borrelia*. 1000X
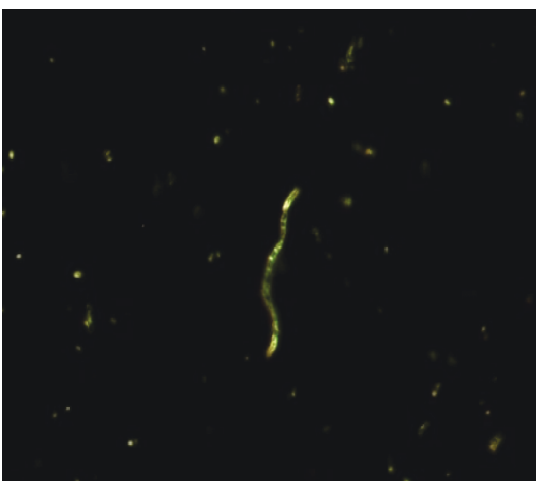
Figure 6A: Darkfield microscopy of dermatological culture fluid from dog C-1 showing motile fluorescent spirochete. 1000X

clinics with unusual filamentous dermatological manifestations of LD. The key diagnostic criterion of a comparable human condition, MD, is the presence of filaments projecting from ulcerative lesions and/or embedded in unbroken skin [14,15]. The filaments associated with MD, which are often vividly colored, are both keratin and collagen in composition and are produced by proliferative activated epithelial keratinocytes and fibroblasts, respectively [14,15]. The blue coloration seen in some filaments is contained within granules and is melanin in composition [15]. Morgellons patients frequently describe changes in hair and other keratinized tissues [13,14]. Sectioned filamentous inclusions in specimens from case C-1 were verified to be of collagen and/or keratin composition, as shown by Gömöri trichrome staining. Nuclei were observed in the shaft of a filament from a specimen obtained from case C-5. The presence of nuclei and the composition of the fibers proves cellular origin and thus the fiber could not be a textile. The specimen was comparable to nucleated filaments seen in human MD dermatological specimens.

As in humans afflicted with MD, the microscopic filaments seen in the canine cases were predominantly white, blue and red. However, in

Table 4: Microscopic findings of dermatological specimens and cultures.

ID	Specimen	Darkfield	Dieterle stain	Bb-immunostain
C-1	Dermatological tissue	N/A	Spirochetes	Positive spirochetes
C-1	Dermatological culture	Spherules, motile spirochetes	Predominantly spherules	Positive spherules
C-2	Dermatological tissue	N/A	Spirochetes	Positive spirochetes
C-2	Dermatological culture	Spherules, motile spirochetes	Spherules	Positive spherules
C-3	Dermatological tissue	N/A	Spirochetes	Positive spirochetes
C-4	Dermatological tissue	N/A	Spirochetes	Weakly positive spirochetes
C-4	Dermatological culture	Spherules, motile spirochetes	Spirochetes, spherules	Positive spirochetes, spherules
C-5	Dermatological tissue	N/A	Spirochetes, spherules	Positive spirochetes, spherules
C-6	Dermatological tissue	N/A	Spirochetes, spherules	Positive spirochetes, spherules
C-7	Dermatological culture	Spherules, motile spirochetes	Spirochetes, spherules	Positive spirochetes, spherules
C-8	Dermatological culture	Spherules, motile spirochetes	Not performed	Not performed
C-9	Dermatological culture	Spherules, motile spirochetes	Not performed	Not performed

Table 5: PCR amplification of canine subject dermatological specimens. All specimens tested positive using nested (N) and/or real time (RT) PCR.

ID	Specimen	Mount Allison University Method and Target	University of New Haven Method and Target	Australian Biologics Method and Target
C-1	Dermatological tissue	(N) OspC	(N) Bb 16S rRNA	(RT) Bb 16S rRNA
C-1	Dermatological culture	(N) OspC	(N) Bb 16S rRNA	
C-2	Dermatological tissue	(N) OspC (N) BB 0006		
C-2	Dermatological culture	(N) OspC (N) BB 0006		(RT) Bb 16S rRNA
C-3	Dermatological tissue	(N) OspC (N) BB 0006	(N) Bb pyrG sequenced – matched Bbss 99%	
C-4	Dermatological culture			(RT) Bb 16S rRNA
C-5	Dermatological tissue			(RT) Bb 16S rRNA
C-6	Dermatological tissue			(RT) Bb 16S rRNA
C-7	Dermatological culture	(N) OspC	(N) Bb 16S rRNA sequenced – matched Bbss 99%	(RT) Bb 16S rRNA
C-8	Dermatological culture			(RT) Bb 16S rRNA
C-9	Dermatological culture			(RT) Bb 16S rRNA

Table 6: PCR amplification of canine control dermatological specimens. All specimens tested negative using nested (N) and/or real time (RT) PCR.

ID	Specimen	Mt Allison University	University of New Haven	Australian Biologics
CC-1	Dander and hair	Negative (N)	Negative (N)	Negative (RT)
CC-2	Dander and hair	Negative (N)	Negative (N)	Negative (RT)
CC-3	Skin biopsy	Negative (N)		
CC-4	Skin biopsy	Negative (N)		

cases C-3 and C-4, in addition to blue and white microscopic filaments, thickened coarse black fibers were also present, and microscopic examination revealed scaling and follicular bulbs consistent with hair structures, thus indicating keratin composition. Dermatological specimens from dogs C-3 and C-4 also displayed deformed follicles. These abnormal hairs and deformed hair follicles were identical to the hair deformities described previously in a human MD patient [12]. Canine MD is complicated by the fact that a dog's undercoat can hide filamentous inclusions or projections. Examination of the skin with a 50X han-dheld microscope under UV light was helpful in identifying canine MD; unlike normal canine hair, the filaments fluoresced and clearly projected up from the skin surface.

In addition to meeting the key diagnostic criterion for MD, the canine cases paralleled human MD in that *Borrelia* spirochetal infection was associated with the filament formation. Spirochetes identified as *Borrelia* spp. were detected in and/or cultured from samples taken from the dermatological lesions of the canine subjects.

Given that these cases demonstrated both ulcerative lesions with unusual filament formation associated with *Borrelia* spirochetal infection, they are entirely analogous to human MD. Since MD-type filamentous lesions are associated with LD in humans, recognition of these lesions could aid in the diagnosis of LD in animals.

In all animals tested in our study, commercial serological testing was negative for Bb. This observation suggests that commercial serological testing may lack sensitivity for accurate detection of LD in dogs. In light of that fact, the diagnosis of Lyme disease in pets should be based primarily on clinical history, such as tick exposure and symptomatology, including lameness, fatigue and behavioral changes. We recommend histological examination with silver nitrate staining and/or anti-*Borrelia* immunostaining to look for spirochetes in cases of canine dermopathy associated with filament formation. Silver nitrate staining and anti-*Borrelia* immunostaining allow for the visualization of spirochetes but cannot determine the identity of visible microorganisms. Consequently, PCR amplification

Borrelia burgdorferi strain B31, complete genome
Sequence ID: gb|CP009656.1| Length: 910687 Number of Matches: 1

Range 1: 588525 to 589180 GenBankGraphics

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
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Query  83     TAATGAGCAAAAATCTAAACCTACTCAACAAAGTGTTAAAACCTTAAATAAAGCAGGTAT  142
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Sbjct  588585 TAATGAGCAAAAATCTAAACCTACTCAACAAAGTGTTAAAACCTTAAATAAAGCAGGTAT  588644
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Sbjct  588765 TATTTATGAAATTCCTATATCTTTTTATAAGCAGGGTGTACATGAGATTTTAAGCTCTAA  588824
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Sbjct  588825 GTTAAATATTAAGGTTGATCCAAAAATAGAAGAGCTTTCAAAGCTTGTAGGAGTTATAAA  588884
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Sbjct  589125 GGCTATTAAATATGCTCGTGAGAATAATATTCCCTTTCTTGGAATTTGTC-TTGGTT     589180
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Figure 7A: Amplicon sequence from dermatological specimen of dog C-3. Forward sequence.

of spirochetal DNA is needed to confirm the genetic identity of spirochetes detected in skin tissue.

In our study, PCR analysis was performed at three independent laboratories in order to avoid possible contamination in a single laboratory and to optimize the PCR results using different techniques. It is important to remember that PCR is not 100% sensitive. We have found that false negative results can occur in clinical specimens because of a number of factors: DNA degradation has occurred, inhibitory substances are present, DNA from competing microorganisms or host DNA may prevent amplification of the target, and the primer may be too specific to detect the genetic diversity of *Borrelia* in clinical specimens. In our experience, PCR amplification and subsequent sequencing from veterinary skin specimens is more challenging than

Borrelia burgdorferi strain B31, complete genome
Sequence ID: gb|CP009656.1| Length: 910687 Number of Matches: 1

Range 1: 588475 to 589128 GenBankGraphics
Alignment statistics for match #1

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Query   328   TAACTTAGAGCTTAAAATCTCATGTACACCCTGCTTATAAAAAGATATAGGAATTTCATA   387
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Sbjct   588528 AATCTCCTGTCTTATTTGTCTTACTGTCTCAATAAATAAAATA-TTTT-CCATATC   588475
```

Figure 7B: Amplicon sequence from dermatological specimen of dog C-3. Reverse sequence.

amplification from human clinical specimens. We attribute this to the fact that there were many other bacteria present in the specimens that were analyzed. High levels of competing microorganisms would complicate the PCR process and decrease the chance of obtaining an amplicon of sufficient quality for sequencing. Only the skin samples from dogs C-3 (Golden Retriever) and C-7 (Miniature Schnauzer) yielded PCR amplicons that could be sequenced, and the DNA from those samples matched *Bb sensu stricto*.

At present we do not know if *Borrelia* spp. are the only bacteria that can trigger MD filament formation. It is possible that other spirochetes such as *Treponema pallidum* or *T. denticola* could induce dermal filament production. *Helicobacter pylori* has been detected in human MD specimens by the research group at the University of New Haven, and Australian Biologics reported finding both *T. denticola* with *Borrelia* spp. in human Morgellons samples. Thus co-infecting pathogens or other spirochetes could be involved in

Borrelia burgdorferi strain B31, complete genome
Sequence ID: gb|CP009656.1| Length: 910687 Number of Matches: 1

Range 1: 445662 to 446067 GenBank Graphics   ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
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Sbjct  445722  AGATTCTTAGCTGCTGCCTCCCGTAGGAGTCTGGACCGTATCTCAGTTCCAGTGTGACCG  445781

Query  127    TTCACCCTCTCAGGCCGGTTACTTATCATTGCCTTGGTAGGCATTTACCCTACCAACTAG  186
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Sbjct  445962  GTAGATCATCCACGCGTTACTCACCCGTTCGCCACTGAATGTATTGCTACATCCCGTTTG  446021

Query  367    ACTTGCATGCTTAAGAAGCACTGCCCA-GTTAGTTCTAAGCCCGGA  411
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Sbjct  446022  ACTTGCATGCTTAAGACGCACTGCCAGCGTTAGTTCTAAGCCAGGA  446067
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Figure 7C: Amplicon sequence from dermatological specimen of dog C-7. Forward sequence.

MD-type filamentous dermatological manifestations. We speculate that tickborne co-infections and co-involved infections such as *H. pylori* and *T. denticola* could be contributing secondary factors in the development of filamentous borrelial dermatitis.

Dogs are susceptible to LD and serve as both disease sentinels and animal models to investigate human tickborne illness [2,3]. For this reason, accurate diagnosis is important to insure (1) that companion animals can receive appropriate treatment and (2) that accurate infection rates will reflect what is happening in the human population. Serological testing coupled with epidemiological and clinical evidence is the principal mode of estimating Lyme disease prevalence in canine populations [2,4,28]. In veterinary practice, the prevailing thought has been that the detection of anti-Bb antibodies may not correlate with clinical disease because of low test specificity, and consequently it is assumed that LD serological testing leads to overestimation of the canine infection rate [2]. The serological results of our study challenge that claim, and canine cases may in fact be under-reported. Some of the dogs in our study were tested by commercially available Lyme serological methods, and all were classified as being serologically negative, yet these dogs had detectible *Borrelia* spirochetes in their skin samples. Our cases illustrate the difficulties with currently available canine LD serological testing, which appear to be similar to the problems with human commercial LD testing [29].

It has been estimated that about 6% of human LD cases are associated with MD [30]. With over 300,000 new human LD cases diagnosed per year in the USA alone [31], MD may prove to be fairly common if increased awareness prompts physicians and veterinarians to look for it. Six of the dogs in our study initially presented at the same veterinary clinic in Alberta, a Canadian province where LD is considered to be extremely rare. The dogs presented to a clinic in which the veterinarians were familiar with the MD literature and included LD in the differential diagnosis. The lack of LD awareness coupled with the misperception that it is rare or non-existent in various geographical areas of the USA and Canada, and compounded by insensitive Lyme serological testing, may cause animal and human LD to go undiagnosed. If MD is proved to be pathognomonic for LD, the dermopathy may be important in assessing the sensitivity of Lyme serological testing in both dogs and humans and may aid in more accurate estimates of infection.

MD lesions have been attributed to delusions of parasitosis or delusional infestation (DI), and many healthcare practitioners are under the impression that the lesions are caused by self-mutilation and implantation of textile fibers [18,19,32]. Recognition of MD in humans as a true somatic illness has met with vehement opposition by the mainstream medical community [18,19,32], even in the face of published evidence demonstrating an association with LD and the detection and isolation of Bb spirochetes from lesions of MD patients [19]. "DI by proxy" and "double DI" are terms that have been introduced to explain cases of suspected MD-like illness in pets presenting at veterinary practices [33]. Just as MD is commonly thought by medical professionals to be a type of DI, filamentous borrelial dermatitis occurring in pets may be misdiagnosed as "DI by proxy" or "double DI" by veterinarians, especially if the pet owner

Borrelia burgdorferi strain B31, complete genome
Sequence ID: gb|CP009656.1|Length: 910687Number of Matches: 1

Range 1: 445623 to 445996GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
675 bits(365)	0.0	373/376(99%)	3/376(0%)	Plus/Minus

```
Query  23      GTGGCGAACGGGTGAGTAACGCGTGGATGATCTACCTATGAGATGGGGATAACTATTAGA  82
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445996  GTGGCGAACGGGTGAGTAACGCGTGGATGATCTACCTATGAGATGGGGATAACTATTAGA  445937
Query  83      AATAGTAGCTAATACCGAATAAGGTCAGTTAATTTGTTAATTGATGAAAGGAAGCCTTTA  142
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445936  AATAGTAGCTAATACCGAATAAGGTCAGTTAATTTGTTAATTGATGAAAGGAAGCCTTTA  445877
Query  143     AAGCTTCGCTTGTAGATGAGTCTGCGTCTTATTAGCTAGTTGGTAGGGTAAATGCCTACC  202
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445876  AAGCTTCGCTTGTAGATGAGTCTGCGTCTTATTAGCTAGTTGGTAGGGTAAATGCCTACC  445817
Query  203     AAGGCAATGATAAGTAACCGGCCTGAGAGGGTGAACGGTCACACTGGAACTGAGATACGG  262
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445816  AAGGCAATGATAAGTAACCGGCCTGAGAGGGTGAACGGTCACACTGGAACTGAGATACGG  445757
Query  263     TCCAGACTCCTACGGGAGGCAGCAGCTAAGAATCTTCCGCAATGGGCGAAAGCCTGACGG  322
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445756  TCCAGACTCCTACGGGAGGCAGCAGCTAAGAATCTTCCGCAATGGGCGAAAGCCTGACGG  445697
Query  323     AGCGACACTGCGTGAATGAAGAAGGTCGAAAGATTGTAAAATTCTTTTATAAATGAGGAA  382
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445696  AGCGACACTGCGTGAATGAAGAAGGTCGAAAGATTGTAAAATTCTTTTATAAATGAGGAA  445637
Query  383     TA-GCTTTTTGTAGGA   397
               || |||||  ||||||
Sbjct  445636  TAAGCTTT--GTAGGA   445623
```

Figure 7D: Amplicon sequence from dermatological specimen of dog C-7. Reverse sequence.

displays symptoms such as anxiety, depression or agitated behavior.

Spirochetal infection in humans can cause neuropsychiatric symptoms that, coupled with strange crawling and stinging sensations with long filaments projecting from skin, leads some people afflicted with MD to erroneously believe they are infested with insects or parasites [20]. Objective consideration must be maintained to achieve an accurate differential diagnosis when an owner afflicted with MD presents a pet with this unusual dermopathy to the veterinarian. When insect infestation and/or parasitosis without evidence of these conditions is proposed by a pet owner as a diagnosis, LD should be considered in the differential diagnosis before giving the pet a diagnosis of "DI by proxy" and dismissing the pet owner as being delusional.

The presence of *Borrelia* spirochetes in MD appears to be a primary etiologic factor for MD development in humans, but the etiology may be multifactorial [13-15]. For example, MD is more frequently seen in middle-aged Caucasian women than in other population groups, so race, gender and age may play an etiologic role [12]. However, the etiology of MD is poorly understood, and secondary etiologic factors remain to be elucidated. Secondary etiologic factors may also play a role in canine skin disease. Although the number of dogs in our study was relatively small, interestingly we had a Golden Retriever, two Schnauzers and four Bulldog-type dogs. Golden and Labrador

Retrievers are known to develop glomerulonephritis associated with LD and therefore may be predisposed to develop a more severe form of tickborne disease compared to other dog breeds [7]. The Schnauzers in our study had light coats that are associated with color dilution gene mutations, which in turn are associated with increased skin sensitivity [34]. Furthermore, Bulldogs and Golden Retrievers are breeds that are genetically predisposed to develop ichthyosis, a dermopathy characterized by rough, dry, scaly skin [35]. In our study group, both male and female dogs were present in equal numbers and in this respect the filamentous dermopathy appears to differ from human MD, where the manifestation appears to be more common in women. The fact that almost all the pets in our study were spayed and/or neutered, however, may account for the fact that there was no female predominance in these animals.

As noted above, human MD occurs in about 6% of patients with LD [30]. This observation suggests that one or more genetic factors may determine the occurrence of the dermopathy in LD patients. The fact that canine MD appears to preferentially affect certain breeds of dogs suggests that similar genetic factors may be important for the development of canine MD in the presence of *Borrelia* infection. Further studies of genomic variation in these animals may help to unlock the mystery of MD in human subjects.

This preliminary study had a number of limitations. No

laboratory method for detecting pathogens in bodily fluids and tissues is 100% sensitive and specific. Therefore, we relied on different methodologies that collectively provided corroborative evidence of *Borrelia* infection in this cohort of dogs. We note that the antibody Abcam ab20950 is known to cross-react with *Treponema pallidum*, *B. hermsii* and *B. parkerii* and may cross-react with *Mycobacterium tuberculosis*; however, we chose this antibody for immunostaining instead of a *Bb*-specific monoclonal antibody because filamentous dermatitis could be caused by one of the *Bb sensu lato* species, or even a treponeme. Using a monoclonal antibody that was *Borrelia* strain-specific could have produced false-negative results. In consideration of the above, the PCR amplification of DNA targets performed in this study provided a more specific method to detect *Borrelia* spp. in clinical specimens, and PCR testing yielded putative or, in the cases of dogs C-3 and C-7, conclusive genetic identifications. In summary, although the immunostaining lacked specificity, the evidence presented by immunostaining was supported by the results obtained by PCR amplification of *Borrelia* DNA targets.

Another limitation of our study was that we had only a small cohort of dogs. In addition, our study does not reflect the geographical distribution of known cases of canine LD. The study was performed in Alberta, Canada, because veterinarians at Heartland Veterinary Clinic in Airdrie, Alberta were familiar with the MD literature and, upon noticing cases in dogs with lesions similar to MD in humans, approached the other authors to collaborate on a study. In view of these sampling and geographical limitations, we acknowledge that some of our observations and conclusions may be open to other interpretations, but the provocative results of our preliminary study suggest that further investigation is warranted.

Conclusions

Analysis performed at five independent laboratories provided compelling, corroborative evidence that a dermopathy comparable to the human condition referred to as Morgellons disease can also occur in dogs. Domestic pets may present at veterinary clinics with filamentous dermopathy associated with spirochetal infection, and this manifestation appears to be analogous to MD in humans. Veterinarians should consider MD or LD in their differential diagnosis when presented with cases of unusual dermopathy that lack other explanations, particularly if unusual filaments are visible in epidermal lesions or are embedded under the skin. Serological testing should not be relied upon for diagnosis of LD. Direct detection of spirochetes in dermatological tissue by histology or PCR amplification of DNA may be more useful than serology to obtain a LD diagnosis in these pets.

Authors' Contributions

MJM participated in the study design and coordination, performed culture, histology and immunohistochemistry experiments and drafted the manuscript. GMR and JLM evaluated the subjects, collected dermatological specimens and wrote the clinical histories. KRF, ES, JB, LM and AF performed PCR testing and DNA sequencing experiments. MCM and RBS participated in the study design and coordination and edited the manuscript. All authors read and approved the final manuscript.

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Author Affiliations   Top

[1]*International Lyme and Associated Diseases Society, Bethesda, Maryland, USA*

[2]*Heartland Veterinary Clinic, Airdrie, Alberta, Canada*

[3]*Department of Biology and Environmental Science, University of New Haven, West Haven, Connecticut, USA*

[4]*Australian Biologics, Sydney, New South Wales, Australia*