INVESTIGATION OF THE MICROBIAL AND MOLECULAR CORRELATES OF MORGELLONS DISEASE

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ABSTRACT

Morgellons Disease (MD) is a rare, mysterious illness that generates controversy in the medical community [1-16]. These contentions stem from difficulties in diagnosis, as MD symptoms overlap with a rare psychiatric disorder, most notably delusional infestation (DI). DI is a fixed, false belief of infestation by animate or inanimate pathogenic entities, despite medical or microbial evidence to the contrary [1-16, 19]. MD patients who exhibit or report lesions containing fibers are often dismissed as having DI, and their physiological symptoms are frequently disregarded. Consequently, MD patients are treated with antipsychotic medications, labeled with DI without an appropriate psychiatric evaluation, and denied examinations for an underlying microbial infection that may cause a filamentous dermopathy [3, 8].

At this time, the etiology and transmission of MD remain unknown, and diagnostic criteria have yet to be established [4, 5, 10, 16]. This is detrimental to patients, as the dispute surrounding MD is substantial [8]. This study aimed to address critical gaps by bridging the methodological limitations of earlier studies, to reduce stigma and skepticism surrounding MD, and to stimulate broader scientific engagement. While this investigation does not establish causality, the study explored the potential associations between pathogens such as *Bartonella henselae*, *Borrelia burgdorferi*, *Helicobacter pylori*, and *Treponema denticola* within MD lesions.

The aim was to support a biological and infectious basis for MD, rather than a solely psychiatric explanation. By using defined clinical criteria for MD, along with reproducible microbial and molecular techniques, this study generated evidence of pathogens presence in affected MD tissue. These findings contribute to a growing body of literature supporting MD as a legitimate physiological condition and challenge the psychiatric-only perspective.

This research may contribute to future clinical protocols; particularly in differentiating MD from psychiatric conditions such as delusional infestation (DI), and promote a more accurate classification, diagnosis, and treatment strategies for MD.

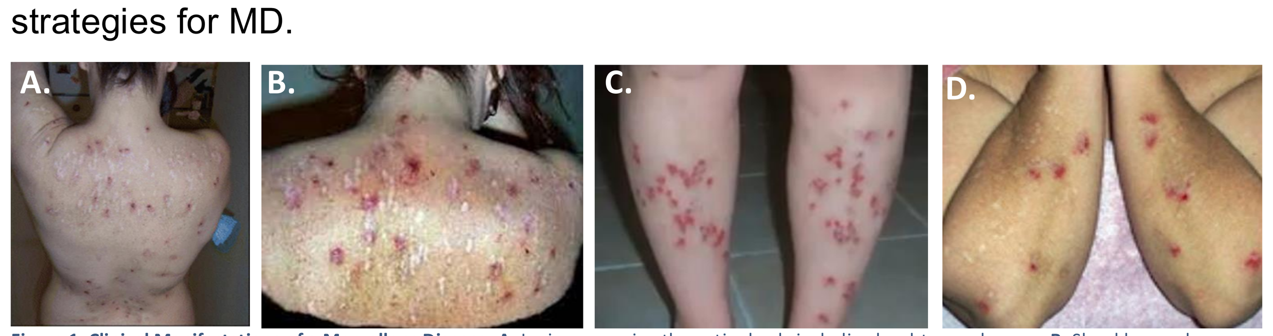

Figure 1. Clinical Manifestations of a Morgellons Disease. **A.** Lesions covering the entire back, including hard-to-reach areas. **B.** Shoulders and upper back showing profuse scarring and lesions. **C.** Front of the legs with multiple open lesions. **D.** Arms with visible scarring and open wounds. Photographs used with full permission. Provided by the Charles E. Holman Foundation. Patient identity has been protected in accordance with ethical standards.

INTRODUCTION

MD is a complex dermopathy, historically regarded as controversial and poorly understood within the medical community [1-16]. A distinct feature of MD is the presence of microscopic and near-microscopic fibers visible within the cutaneous and subcutaneous layers of the skin [1-5, 7-11, 13, 17-19]. These fibers range in a multitude of colors, including red, blue, black and white, and are accompanied by unusual filamentous inclusions and projections found in spontaneous, slow-healing, or non-healing lesions [1-5, 7, 9-11, 13-18, 20]. Although the origin of fiber coloration is not fully understood, fiber-like filaments are perceived to result from the overproduction of keratin and collagen, with blue filaments containing granules of melanin [3, 7-11, 15, 17, 18]. Analyses from past research have yielded varied causations to production [2, 3, 5-8, 16, 18]. MD does not discriminate, affecting individuals regardless of age, gender, race, or background [6]. The signs and symptoms of MD include skin lesions, crawling sensations, fatigue, cognitive difficulties, behavioral effects, and fibers [1, 3, 11, 15, 19]. Additional symptoms may include Lyme-like manifestations such as musculoskeletal, neurological, and cardiovascular involvement [3, 8-11, 14-16].

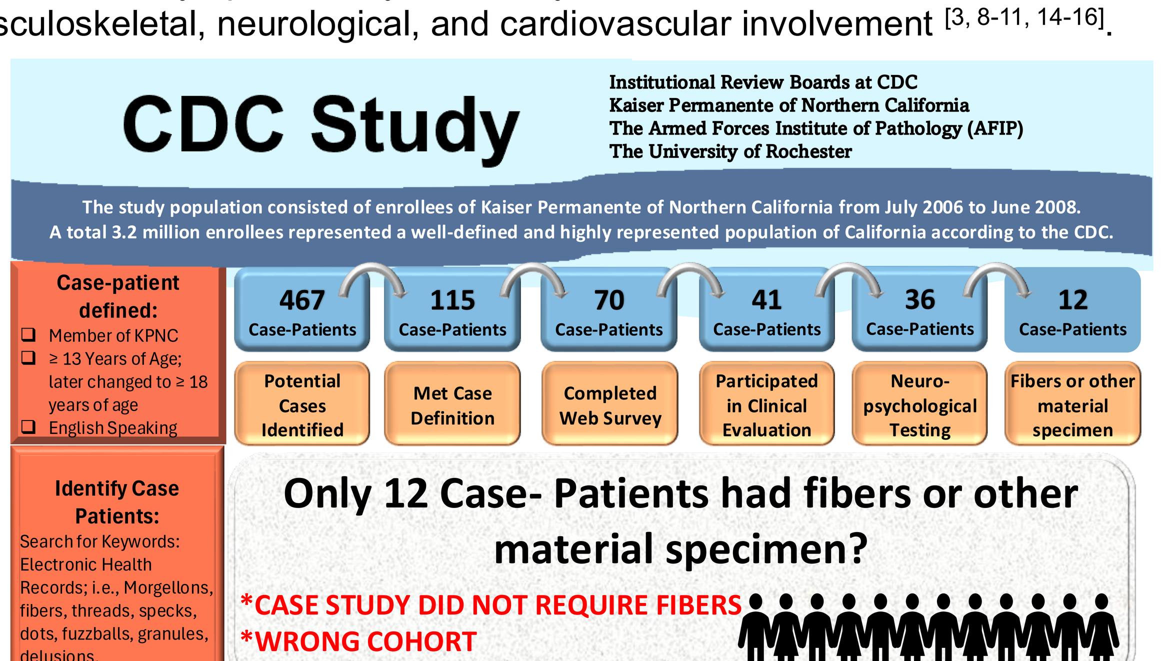

Figure 2. The 2012 CDC Investigation.

METHODS

SAMPLE POPULATION

- 115 MD epithelial tissue samples (n=115) were obtained from primary care physicians, internal medicine specialists, and direct patient submissions across the United States.
- All samples met the case definition for MD that required the presence of multicolored fibers, including red, blue, black and white that were embedded in and/or projecting from epithelial tissue samples.
- Healthy epithelial tissue samples were used as negative controls.

MOLECULAR TECHNIQUES

Microscopy

Epithelial tissue samples were examined for the presence of multicolored fibers using a stereomicroscope and a compound microscope.

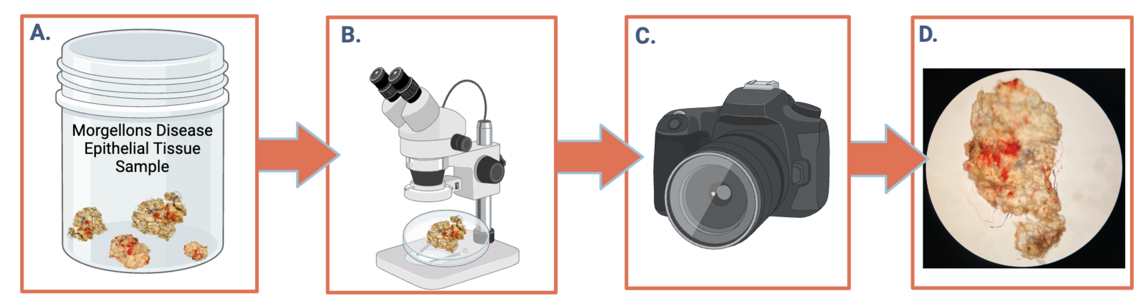

Figure 3. Sample Processing and Imaging Workflow. A. Epithelial tissue samples are stored in airtight containers. **B.** Each sample are visualized for the presence of multicolored fibers. **C.** Each sample is imaged. **D.** All images are cataloged and assigned an identifying number.

DNA Extraction

Cryogenic Preservation and Pulverization was performed.

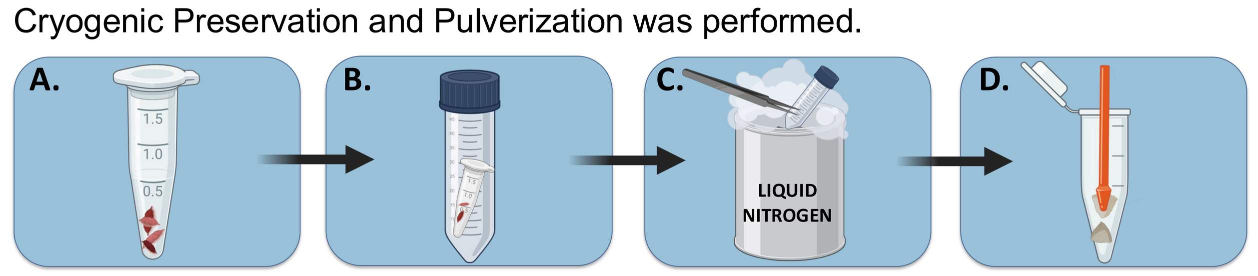

Figure 4. Cryogenic Preservation and Pulverization. A. Epithelial tissue samples are placed in a 1.5 mL microcentrifuge tube. **B.** 1.5 mL microcentrifuge tube is placed in a 50 mL conical tube. **C.** Conical tube is place in the liquid nitrogen for 5 minutes or until the tissue has turned white. **D.** Pulverization with a pestle breaks down the dense tissue, breaking open cells and prevents DNA degradation.

DNA extraction was performed using three commercial kits:

- Monarch® Genomic DNA Purification Kit
- E.Z.N.A. Tissue DNA Extraction and Purification from Tissue Kit
- QIAGEN DNeasy Blood & Tissue Kit

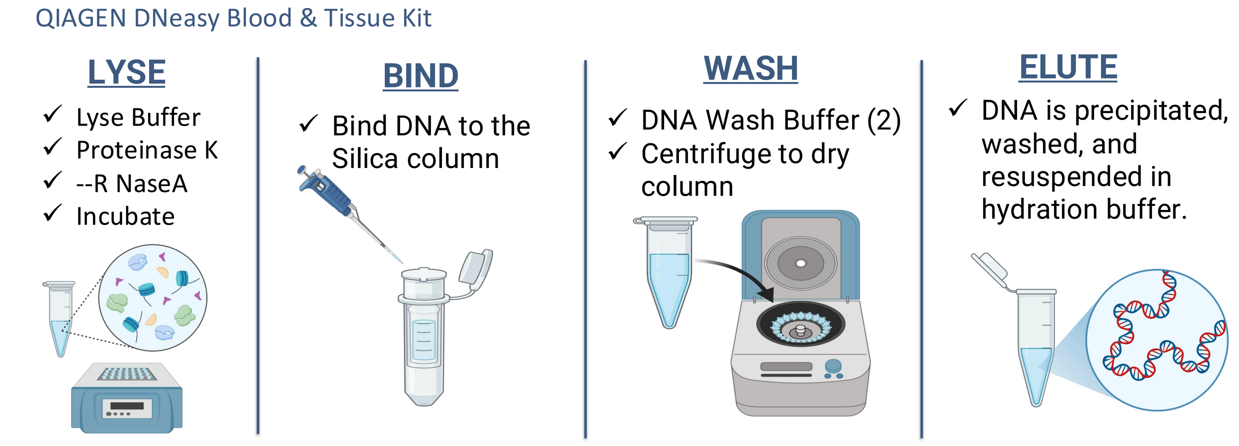

Figure 5. DNA Extraction Process. All commercial kits were followed according to the manufacturers' recommendation. Created with BioRender.com

Polymerase Chain Reaction

PCR was used to amplify our gene of interest:

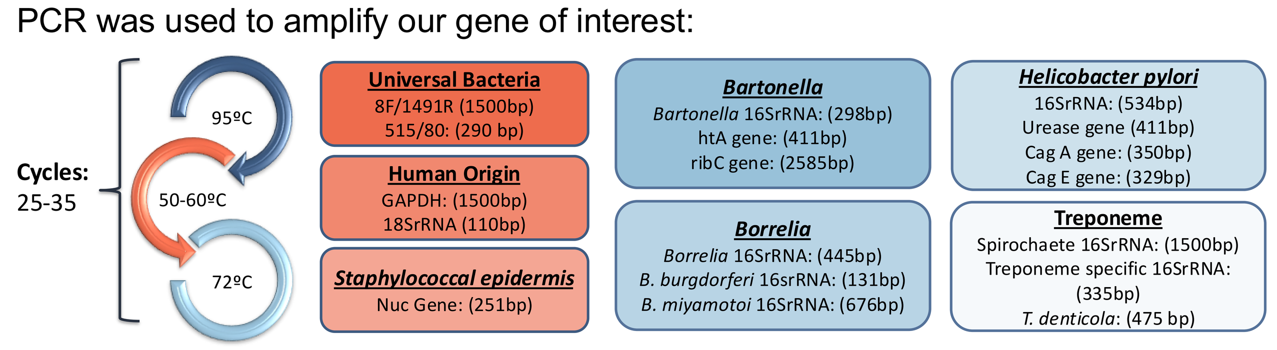

Figure 6. Conventional PCR for Amplification of DNA. Extraction Process. Universal primers were employed to assess DNA integrity, following host identity to ensure the sample was human derived, and from the skin. The DNA was then assessed using our bacterial primers of interest. PCR was conducted on three different thermocyclers: Techne TC-3000 Thermal Cycler (Techne, UK), Mastercycler® Nexus Thermal Cycler (Eppendorf, Germany), and SimpliAmp™ Thermal Cycler (Applied Biosystems™, Thermo Fisher Scientific, USA). Go Taq® Colorless Master Mix (Promega) were followed according to the manufacturer's recommendations for the cycling conditions.

Gel Electrophoresis & Purification

A 2% agarose gel ran at 75 Volts for 2 hours and stained with SYBR Safe DNA Stain for 15 minutes and viewed on a BIO-DOC. Positive bands were excised and purification preformed using the QIAquick® Gel Extraction Kit.

NanoDrop Spectrophotometer

The DNA concentration was quantified using 1-2µl of DNA.

Sanger Sequencing

DNA was sent to OSU's core facility for Sanger Sequencing with subsequent bioinformatic analysis.

Bioinformatic Tools

Sequence were analyzed using Finch TV for chromatogram visualization and trimming of trace data. Nucleotide sequences were then interpreted, verified, and aligned using the NCBI BLAST (Basic Local Alignment Search Tool) for sequence comparison and validation.

RESULTS

All samples were visualized and met the case definition of MD.

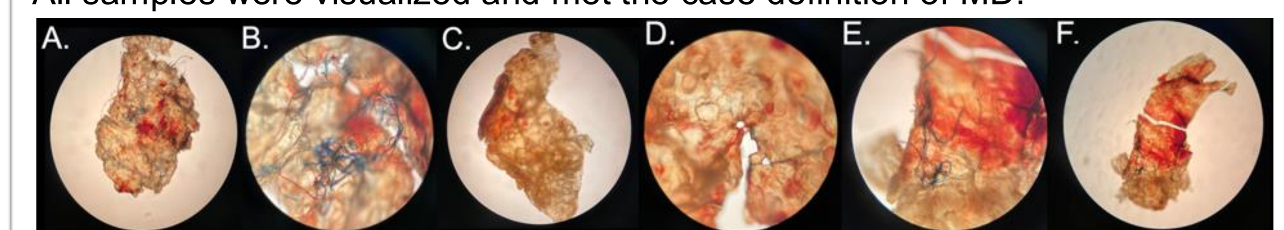

Figure 7: Micrograph Images of Fibers. Samples A, C, and E were scanned at 40x magnification to capture the entire lesion. Samples B, D, and E were imaged at 100x magnification. All samples showed the presence of fibers in red, blue, black, and white. Images were taken by Samantha Rice at the Center for Investigation of Morgellons Disease Laboratory on 01/28/22.

PCR products were visualized and positive bands extracted for purification.

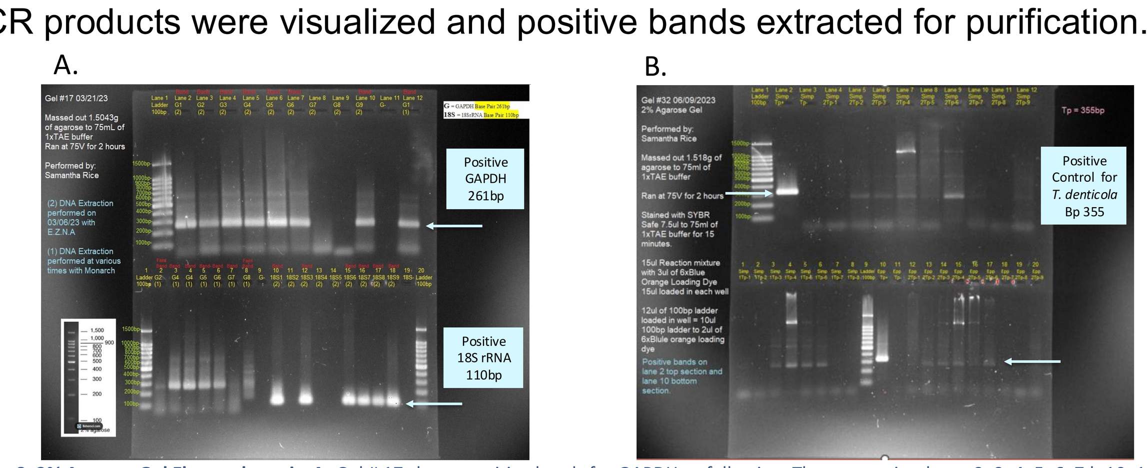

Figure 8. 2% Agarose Gel Electrophoresis. A. Gel # 17 shows positive bands for GAPDH as following: The top section lanes 2, 3, 4, 5, 6, 7,b 10, 12 and bottom section with lanes 2, 3, 4, 5, 6, 7, 8 and the 18S rRNA were positive in lanes 10, 12, 16, 16, 17, and 18. In gel 17 the positive controls G9 were the positive controls and the negative controls were negative. B. In Gel # 32 positive bands for *Treponema denticola* were in the top section with lanes 2, 5, 6, 7, 8, 9, 10, 12 and in the bottom section lanes 3, 4, 5, 6, 10, 13, 14, 15, 16, 17. Positive bands were indicated at 355bp.

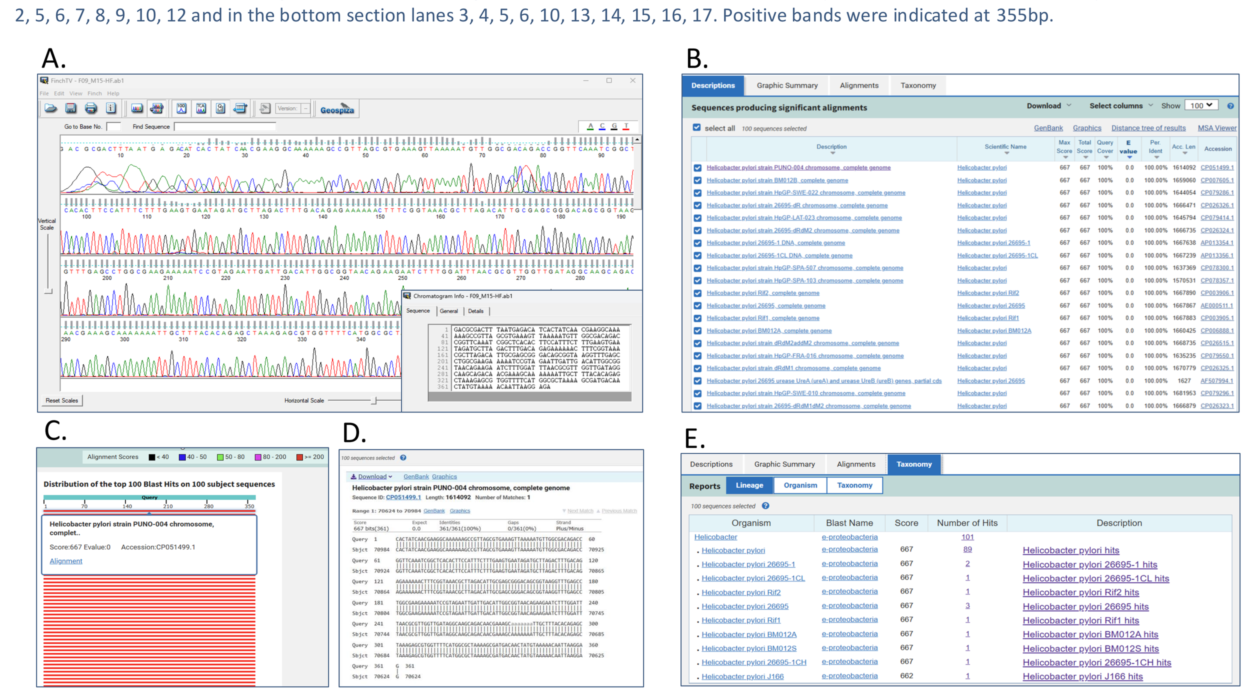

Figure 9. Sanger Sequencing and Bioinformatic Tools. A. Trace files from Sanger Sequencing data was generated for Sample M15 using *Helicobacter pylori* 16S rRNA primers at the OSU Stillwater Core Facility. The forward and reverse chromatograms were visualized in Finch TV, and the noise at the beginning and ends of each chromatogram was trimmed. **B.** Nucleotide BLAST analysis of M15 forward and reverse sequences showed the closest match to *Helicobacter pylori* strain PUNO-004 chromosome, complete genome. The query cover was 100% with 100% per identity, indicating a perfect match. The E-value was 0.0, suggesting a highly significant alignment unlikely to have occurred by random chance. **C.** The distribution of the top 100 Blast Hits shows sequences with the highest similarity or alignment scores, with the first hit aligning with *Helicobacter pylori* strain PUNO-004 chromosome, complete genome. **D.** The top alignment exhibited 100% with 0/361 gaps (0%). **E.** A total of 101 hits matched with *Helicobacter* species.

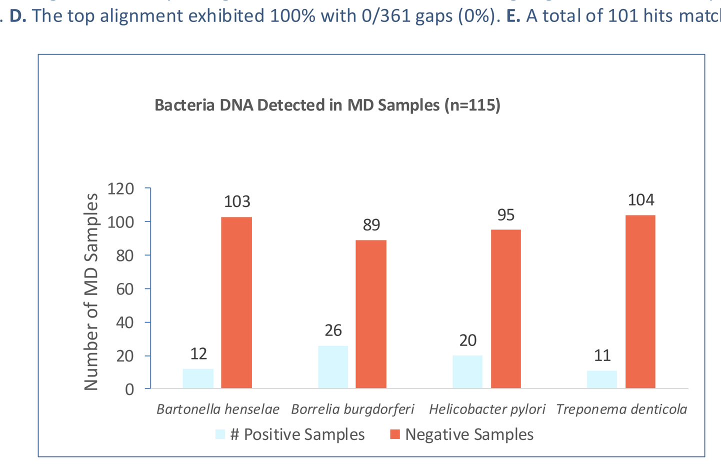

Figure 10. Bacterial DNA Detected by PCR and Agarose Gel Electrophoresis in MD Samples (n=115). The number of samples positive and negative for each bacterial species was determined using PCR and confirmed by gel electrophoresis. Data are presented as the number of samples detected for *Bartonella henselae*, *Borrelia burgdorferi*, *Helicobacter pylori*, and *Treponema denticola*.

Table # 1. Positive Samples Successfully Sequenced

ORGANISM IDENTIFIED	POSITIVE SAMPLES SEQUENCED
Bartonella henselae	2
Uncultured Bartonella sp.	1
Bartonella quintana	1
Borrelia burgdorferi	8
Helicobacter pylori	11
Treponema denticola	2
Uncultured Treponema	1
Treponema sp.	1

Table # 2. Coinfection within Sample Successfully Sequenced

COINFECTIONS	SAMPLE ID MATCHED
Bartonella henselae & Helicobacter pylori	1
Bartonella henselae & Borrelia burgdorferi	1
Bartonella quintana & Borrelia burgdorferi	1
Helicobacter pylori & Treponema denticola	1

Table #3. American Type Tissue Collection (ATCC) Positive Controls.

Positive Control	ATCC
Bartonella henselae	Strain Houston-1; ATCC 49882D-5
Borrelia burgdorferi	Strain B31; ATCC 35210D-5
Helicobacter pylori	Strain 26695; ATCC 700392D
Treponema denticola	Strain a; ATCC 35405D-5

Table #4. Healthy Tissue and Environmental Controls

Sample ID	*Bartonella henselae*	*Borrelia burgdorferi*	*Helicobacter pylori*	*Treponema denticola*
Healthy Controls #1-9	Negative	Negative	Negative	Negative
Hairball	Negative	Negative	Negative	Negative
Dryer Lint	Negative	Negative	Negative	Negative
Centrifuge (DNA only)	Negative	Negative	Negative	Negative
Centrifuge (PCR only)	Negative	Negative	Negative	Negative
PCR Micropipettes	Negative	Negative	Negative	Negative
Main Bench Surface	Negative	Negative	Negative	Negative
Autoclave	Negative	Negative	Negative	Negative
Women's Bathroom	Negative	Negative	Negative	Negative
Stairwell (Main)	Negative	Negative	Negative	Negative
Water Fountain	Negative	Negative	Negative	Negative
ATCC Positive Control	Positive	Positive	Positive	Positive
Nuclease Free Water	Negative	Negative	Negative	Negative

CONCLUSION

- The MD case definition for this study, required the presence of multicolored fibers, including red, blue, black and white that were embedded in and/or projecting from epithelial tissue samples.
- This study addressed limitations of earlier research, including the 2012 CDC investigation, which lacked strict case definitions and few fiber-positive subjects. Previous studies (e.g., Middelveen et al., Fesler et al.) also faced skepticism due to limited reproducibility and reliance solely on gel electrophoresis. Our study addressed those limitations by using DNA sequencing to confirm pathogen identify at the nucleotide level.
- DNA analysis, PCR, and Sanger sequencing specifically targets biological material, ensuring that neither natural fibers, nor synthetic fibers were detected, thereby excluding textile fibers. Natural fibers are composed of cellulose or protein, while synthetic fibers are petroleum-based polymers, and both do not contain intact genomic DNA that can be amplified or sequenced by our pipeline.
- DNA from bacterial pathogens were identified in MD tissue samples, including *Bartonella henselae*, *Borrelia burgdorferi*, *Helicobacter pylori*, and *Treponema denticola*.
- We emphasize reproducibility in our methods, which are supported by rigorous controls at every stage throughout the research process.
- Although co-infections complicated the identification of a single possible causative agent, the consistent detection of these pathogens in MD lesions suggests an infectious basis that warrants further investigation.
- DNA from MD epithelial tissue samples was sequenced and identified using the NIH genetic sequence database, GenBank®, to ensure the results were conclusive and scientifically valid, addressing a major criticism of earlier research.
- This study provided strong evidence that MD is associated with infectious agents and has a biological basis, rather than being solely psychiatric in origin.
- Bovine digital dermatitis (BDD) supports a model in which MD lesions may result from chronic infection, bacterial sequestration, and persistent inflammation, as BDD involves similar pathogenic mechanisms including *Treponema* species and filament formation.
- While our experimental relevance was aimed towards MD, these findings have broader implications for dermatology and infectious diseases.

FUTURE RESEARCH

- Further research is needed to continue exploring the etiology, support our findings, and replicate these results.
- Design additional primers to cover bacterial genetic diversity.
- Investigate MD fiber pigmentation by designing primers for bacterial pigment genes to explore whether fiber colors are produced by bacterial agents or result from the host's physiological response.
- Incorporate primers to detect biofilm related genes in MD clinical samples to determine whether biofilm formation contributes to persistent, chronic infections observed in slow or non-healing lesions of MD patients.
- Use next-generation sequencing and metagenomics to investigate the microbiome and bacterial viability, providing insight into the microbial communities involved.

REFERENCES

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