# Molecular Analysis of the Systemic Dermatoses of Morgellons Disease

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## ABSTRACT

Morgellons disease (MD) is a multisystem infectious disease historically viewed as controversial and poorly understood by the medical community.[1,2,3,4,6,8] These contentions involve difficulties in diagnosis, as symptoms of MD have similarities consistent with a psychiatric disorder involving the false beliefs of infestation by parasites, also known as delusional parasitosis or delusional infestation.[1,2,3,6] Currently, the factors determining the etiology and transmission of MD are still unknown and the dispute surrounding Morgellons is substantial.

The aim of this study is to investigate if an infectious etiology of systemic dermatoses is present.

- ❑ Epithelial tissue samples are collected and deidentified remaining anonymous to researchers.
- ❑ Molecular biology tools are implemented for the detection of unusual microbial organisms, specifically, *Bartonella henselae*, *Borrelia burgdorferi*, *Helicobacter pylori*, and *Treponema denticola.*
- ❑ Previous research endeavors suggest MD is a disorder associated with a tick-borne illness, caused by the bacterium *Borrelia burgdorferi,* a pathogenic spirochete and the causative agent of Lyme disease (LD).[8,15]
- ❑ MD studies are currently focused on spirochetes as the causative agent to understand if a coinfection exists and/or if multifactorial etiology is a cohort.
- ❑ We hypothesize the presence of multiple pathogenic organisms found in dermatological specimens suggesting infectious pathogens in MD etiology.
- ❑ Recognition of the potential coexistence of multiple pathogens in MD etiology may stimulate the development of novel approaches to diagnosis and treatment. Therefore, allowing multiple diagnostic approaches to be applied simultaneously to detect for the major pathogens in MD.

## INTRODUCTION

A history of MD can be found in many documented cases dating as far back as the 1600s with the first report in the United States recorded in 2002. Coined by Sir Thomas Brown, an English physician, “The Morgellons” was derived from “mouscouloun” from the Latin term Muscula, meaning “a little fly”.[1,2,3,20,21] MD doesn’t discriminate – it affects people of all ages, genders, and ethnicities. In MD patients, a distinct feature of near-microscopic and microscopic fibers is visual within the dermopathy, possessing unique characterizations and formations within the cutaneous and subcutaneous layers of the skin.[1,2,3,4,6] These unique fibers are spontaneous and consist of a multitude of colors.[5,7,8] Although the color of fibers is not fully understood, fiber-like filaments are perceived to be caused by an overproduction of keratin and collagen, with blue filaments containing granules of melanin.[5,8] However, analysis from past research has given mixed results from unknown to keratin. Other signs and symptoms of MD include crawling sensations on and under the skin, intense itching, severe fatigue, cognitive difficulties, and behavioral effects.[3,4,6] Recognition of the potential coexistence of multiple pathogens in MD etiology may stimulate the development of novel approaches to diagnosis and treatment. Therefore, allowing multiple diagnostic approaches to be applied simultaneously to detect for the major pathogens in MD.

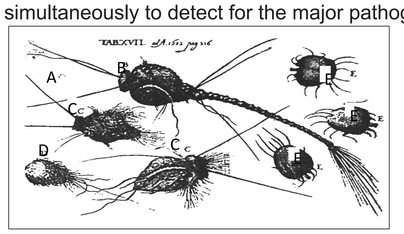



**Figure 1.** Sir Thomas Browne Reproduction of Parasites. A. Image of parasites under the naked eye; **B, C, D,** under the microscope, **B** alone being a perfect specimen. **E.** Figures of *Cirones* or *Sarcoptes hominis.*

## METHODS

### Molecular Techniques

### ❑ Microscopy

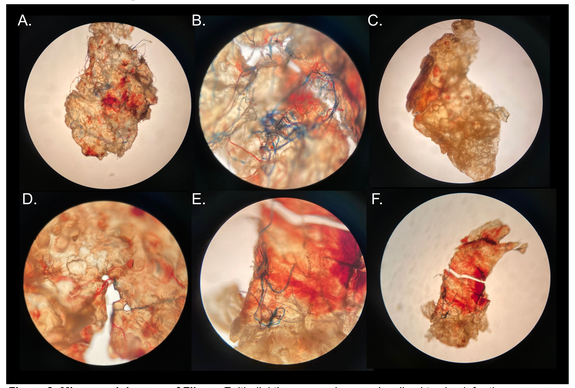


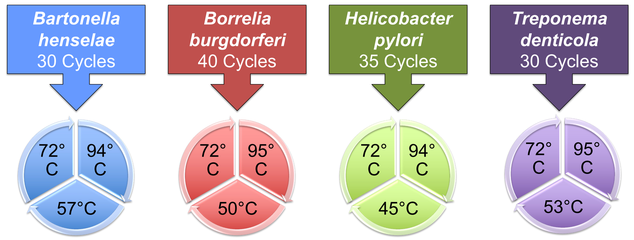

**Figure 2: Micrograph Images of Fibers.** Epithelial tissue samples are visualized to check for the presence of fibers. Sample A., C. E. is scanned at 40x to visualize the whole lesion sample. Sample B., D., and E. are magnified at 100x. All samples show the presence of fibers ranging from blue, red, and black in color. Images were taken by Samantha Rice in the Center for Investigation of Morgellons Disease Laboratory on 01/28/22.

### ❑ DNA Extraction

DNA extraction is performed using a kit by Monarch® HMW DNA Extraction Kit for Tissue.

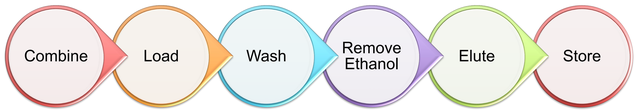
- Homogenize → Lyse → Separate → Precipitate → Wash x 2 → Dissolve → Elute

### ❑ Polymerase Chain Reaction




### ❑ Gel Electrophoresis


### ❑ PCR and Gel Purification




### ❑ NanoDrop Spectrophotometer

Measuring tool used to quantify the DNA concentration using 2µl of sample on a pedestal.

### ❑ DNA Sequencing

DNA is sent to OSU’s core facility for Sanger Sequencing with subsequent bioinformatics analysis.

### ❑ Bioinformatic Tools

Bioinformatic tools are implemented, and nucleotide sequences interpreted for verification of our genes of interest.

## RESULTS

- ❑ Images of 2% Agarose Gel with Subsequent Controls

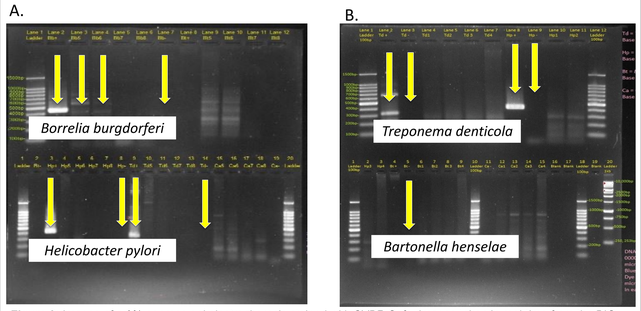



**Figure 3:** Images of a 2% agarose gel electrophoresis stained with SYBR Safe. Images taken in real-time from the BIO-RAD Gel Imager. **A.** Images shows faint bands of *B. burgdorferi* in top lanes 3 and 4, positive controls in lane 2, and negative control in lane 7. In the bottom section, *H. Pylori* positive control in lane 3 with negative control in lane 8. Positive control for *T. denticola* in lane 9 and negative control in lane 14. **B.** Positive control of *Treponema denticola* in lane 2 with negative control in lane 3 in the top section. Positive control of *Helicobacter pylori* in lane 8 and negative control in lane 9. Lane 5 in the lower section shows negative band for *Bartonella henselae.* Positive control for *Bartonella henselae* is negative as expected, new control was recently ordered.

- ❑ **National Center Biotechnology Information BLAST Nucleotide Results**

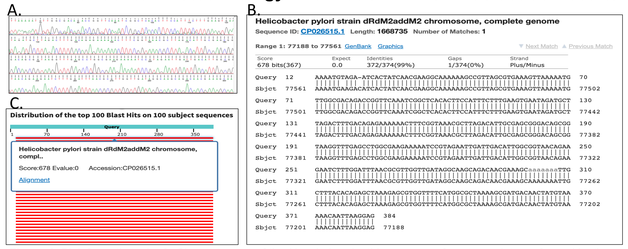



**Figure 4:** Bioinformatic tools. **A.** Chromatogram of the *H. pylori* nucleotide sequence from trace data performed by the Sanger DNA Sequencing viewed on FinchTV. **B.** The distribution of 100 blast hits on the query sequence for the *H. pylori* strain. **C.** Sequence ID from the number one match in GenBank producing significant alliances for the *H. pylori* strain dRdM2addM2 chromosome.

### ❑Preliminary Research → 32 Samples Pending

**Table 1:** Positive microbial organisms found in MD epithelial tissue samples.

| Bacteria of Interest | Positive DNA Samples | Positive Identification in NCBI Blastn |
|---|---|---|
| *Bartonella henselae* | #1, BIF, BM2, 1E, 1F, 1G, 1P, 1U, 1W, 1X, 1Y, 2O, 4A, 4E, 4H, B1, B2, B6, BIF, BOR1, BIG, 1, 1A , 6 | B6, 1U, 6 |
| *Borrelia burgdorferi* | B1, B2, B6, BIF, BOR1, BIG, #1, 1A, 1B, 1C, 1F, 1G, 1J1, 1K, 1M, 1U, 2B, 1, 4A, 4B, 4B1, CC, 4D, 4D1, 4E | #1, 1F, 2B, 1, 1U, Bif, Bor1, B1, B2 |
| *Helicobacter pylori* | BM2, H1L, H17, H9, 1, 1E, 1G, 1K, 1L, 1P, 1U, 1X, 1Y, 2L, 2M, 2N, 2O, M15, M17, HM2, HM1, H17, Hil | M15, M17, 1E, 1G, BM2, 2L, 1Y, H17, 2O, HM2, HM1, H17, 1L, Hil |
| *Treponema denticola* | BM2, T2, TMI, T4, 3S, TN6, 16W, P35, TP4, T1, 1, 2, 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1K, 1L, 1M, 1O, 1P, 1R, 1S, 1U, 1V, 1W, 1X, 1Y, 1Z, 2F, 2K, 2L, 2M, 2P | T4, TN6, 1M |

**Table 2:** Environmental Controls

| Environmental Controls | *Bartonella henselae* | *Borrelia burgdorferi* | *Heliobacter pylori* | *Treponema Denticola* |
|---|---|---|---|---|
| Epithelial Tissue Sample #1 | Negative | Negative | Negative | Negative |
| Epithelial Tissue Sample #2 | Negative | Negative | Negative | Negative |
| Epithelial Tissue Sample #3 | Negative | Negative | Negative | Negative |
| Epithelial Tissue Sample #4 | Negative | Negative | Negative | Negative |
| Autoclave | Negative | Negative | Negative | Negative |
| Women’s Bathroom | Negative | Negative | Negative | Negative |
| Main Stairwell | Negative | Negative | Negative | Negative |

*All environmental controls are negative.*

## CONCLUSION

- ❑ Our results indicate the presence of multiple pathogenic organisms found in dermatological specimens, suggesting an infectious etiology of the dermopathy is present.
- ❑ DNA analysis *of B. henselae, H. pylori, B. burgdorferi*, and *T. denticola* have successfully matched with the GenBank®, the National Institute of Health genetic sequence database.
- ❑ In preliminary research, over 95 molecular epithelial tissue samples were analyzed with each bacteria of interest; 109 positive bands were extracted with 29 successful sequences.
- ❑ Over 32 molecular epithelial tissue samples have been analyzed, with results pending at this time.
- ❑ Environmental contaminant controls were implemented to verify these bacterial agents are not readily found in the environment.
- ❑ Although purification from PCR yields a higher concentration of DNA, gel purification yields a higher purity.
- ❑ Research suggests there may be an infectious etiology of the dermopathy is present.
- ❑ Through further analysis, the potential coexistence of multiple pathogens in MD may hold the answers to this unsolved mystery and bring a better understanding for MD to be recognized in the medical community.

## FUTURE RESEARCH

- ❑ Preliminary research is currently focused on analyzing Oklahoma Ticks for pathogenic organisms found in MD epithelial samples.
- ❑ The identification of a pathogenicity island is currently under investigation, preliminary research has shown the presence of genetically unique variants.
- ❑ Future research needs to be conducted to continue the exploration of etiologic causes to support our findings.
- ❑ Replication of preliminary data is ongoing for the credibility of scientific claims.
- ❑ Additional primers are currently being assessed to assay in future samples.

## REFERENCES

Scan me!

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