


Is This Zebra Really a Zebra? The Challenge of Diagnosing Rare Fungal Infections in Veterinary Pathology

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Shawn R. Lockhart¹ and Joy M. Gary² 

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The diagnosis of unusual fungal infections poses a challenge to the veterinary pathologist for a variety of reasons, including the lack of specific tests for many agents, the bewildering array of possible environmental contaminants, and the general lack of exposure to unusual fungal cases in daily practice. This challenge is highlighted by More et al,¹³ published in this issue of *Veterinary Pathology*. The authors describe a series of mycotic rhinitis and sinusitis cases in horses in Florida, including the steps taken to identify the specific agents responsible and the interesting and unique features of these agents and infections. The challenges associated with identifying these agents by polymerase chain reaction (PCR) and the unique features of some fungi are highlighted in that article.

While some of the more common fungal agents, such as *Coccidioides*, *Candida*, *Cryptococcus*, and *Aspergillus*, can be diagnosed either morphologically or with easily accessible reagents (special stains or specific antibodies), most of the less frequently encountered fungi pose a diagnostic challenge. The diagnosis and characterization of fungal infections are of increasing importance, as more resistant strains are emerging and fungal agents can be ubiquitous in the environment.^{16–18} However, because morphology can be challenging and can vary within fungal species, PCR is increasingly relied upon for identification of the agent responsible for the infection.^{2,5,11}

While culture is the preferred method of diagnosis of fungal infections when fresh tissue is available, PCR from formalin-fixed, paraffin-embedded tissue (FFPE-PCR) is a tool used by many clinical microbiology reference laboratories, including the Centers for Disease Control and Prevention, for diagnosis of fungal cases where culture is not available or where no organisms are grown in culture.¹⁴ Some rare and interesting diagnoses can be made with this method when other forms of identification do not produce results.^{1,15,19} However, caution needs to be observed in the interpretation of FFPE-PCR results because, as More et al¹³ pointed out, this methodology can lead to erroneous results, which become apparent when the species identified by PCR does not match the morphology seen in the tissue or the culture results.¹¹

Erroneous PCR results are most frequent in specimens from nonsterile body sites such as the nasal cavity. As fungal spores are

ubiquitous in the environment and animals, with their noses to the ground, are constantly filling their sinuses with these spores, any species detected by PCR from nasal/fungal specimens may simply represent an environmental contaminant and not the cause of an infection. Notably, it is not just fungal spores in the air that cause false-positive FFPE-PCR results; fungal DNA is also ubiquitous in the environment and has been observed in blood collection tubes, primers, PCR reagents and master mixes, lyticase, and other solutions.^{3,7,12} For these reasons, caution is needed when interpreting FFPE-PCR results, especially when a fungal species not previously known to cause infection is identified.

To help mitigate possible false-positive results, choosing a strong database to identify the sequences that result from FFPE-PCR is very important. In their article, More et al¹³ used the National Center for Biotechnology Information (NCBI) database, which is used most widely by both those depositing a sequence and those wishing to identify an unknown sequence. However, not all sequences, such as the fungal 28 S ribosomal DNA (rDNA) sequence, are equally represented and curated within the database,⁴ which can lead to either an erroneous identification or a less stringent nucleotide match.¹³

The current standard from the Clinical and Laboratory Standards Institute for sequence identity of bacteria and fungi (Standard MM18E2) does not provide guidance on what percent nucleotide sequence match is required to assign a genus or species name. However, there are other sources of recommendations.⁹ In a recent publication on sequence-based identity of over 9000 yeast, it was determined that a threshold of $\geq 98\%$

¹Mycotic Diseases Branch and Infectious Disease Pathology Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

²Infectious Disease Pathology Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

Corresponding Author:

Joy M. Gary, Infectious Diseases Pathology Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H18-SB, Atlanta, GA 30329, USA.
Email: joy.gary@cdc.hhs.gov

sequence identity was good for accurate species identity and $\geq 96\%$ was good for genus identity.²¹ There is no similar guidance for molds, but the yeast thresholds would most likely serve as a liberal proxy.

Another difficulty of fungal diagnosis often confronting pathologists is the unique vocabulary associated with fungi and fungal structures in tissue. Terms like *blastoconidia*, *chlamydoconidia*, and *pseudohyphae*, along with *ribbon-like hyphae* or *acute angle branching*, are specific to certain fungi. These terms are classically descriptive for a pathologist but can be inaccurate when applied to the wrong agent, causing confusion for clinicians. An excellent review by Guarner and Brandt⁶ on histopathologic diagnosis of fungal infections is a helpful resource and should be on the desktop of any pathologist who may diagnose fungi in tissue. This or a similar review will allow pathologists and clinicians to familiarize themselves with the correct descriptive terms when making a fungal diagnosis.

With this spectrum of challenges facing the diagnostician, perhaps one of the hardest questions for a pathologist to answer in the face of an unusual fungal case is, “How far do I need to go with this diagnosis?” It is important to remember that the primary purpose of diagnosis is facilitation of treatment of the patient and prevention of further cases. Because the available antifungal agents for invasive infection are essentially limited to 2 classes, azoles and polyenes, the assignment of fungi to broad classes may be acceptable to facilitate treatment. For example, diagnosis of mucormycosis (the term *zygomycete* is no longer taxonomically valid⁸), phaeohyphomycosis (dark molds), hyalohyphomycosis (hyaline, nonpigmented molds), or yeast may provide sufficient information for the veterinarian to treat the patient. However, species-level diagnosis could be helpful, especially for epidemiology, in situations where multiple animals are affected or emergence of a new agent is suspected. For example, species-level diagnoses allowed us to identify the recent *Ophidiomyces ophidiicola* emergence in snakes¹⁰ and it was an astute veterinarian who first noticed the increasing number of cases of cryptococcosis in the Pacific Northwest that led to the identification of the emergence of *Cryptococcus gattii* in animals and humans.²⁰

Animals, by nature, have close contact with their environment, which exposes them to an unusual array of fungal pathogens and contaminants that are uncommon in clinical practice. As such, the diagnosis of unusual fungal infections can be challenging and should be approached with caution, as FFPE-PCR, terminology, and morphology can all be misleading. Interpretation of molecular and special stain results should be made in context of each individual case, with knowledge of the limitations of each assay, and these limitations should be clearly communicated.


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ORCID iD

Joy M. Gary  <https://orcid.org/0000-0002-0483-6986>

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