ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2019, 57, 256–259
doi: 10.1093/mmy/myy003
Advance Access Publication Date: 16 February 2018
Brief Report

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Phylogenetic analysis of *Histoplasma capsulatum* var *duboisii* in baboons from archived formalin-fixed, paraffin embedded tissues

M. Hensel[1,*], A. Rodrigues Hoffmann[1], M. Gonzales[2], M. A. Owston[2] and E. J. Dick Jr.[2]

[1]Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas, USA and [2]Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, Texas, USA

*To whom correspondence should be addressed. M. Hensel, DVM, Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas, USA. Tel: 979-845-4654; E-mail: mhensel@cvm.tamu.edu

Received 18 October 2017; Revised 21 December 2017; Accepted 5 January 2018; Editorial Decision 1 January 2018

Abstract

Histoplasma capsulatum var. *duboisii* (*Hcd*) infections have been well documented to cause chronic granulomatous disease, mainly involving the skin of baboons and humans in African countries primarily. This retrospective study classified the subspecies of *Histoplasma* and developed a phylogenetic tree utilizing DNA sequences extracted from formalin-fixed, paraffin embedded (FFPE) tissues from 9 baboons from a research colony in Texas histologically diagnosed with *Hcd*. Based on sequence analysis of ITS-2, Tub-1, and ARF, *Hcd* isolated from the archived samples closely aligns with the African clade and has 88% sequence homology with a sample isolated from an individual in Senegal.

Key words: *Histoplasma capsulatum* var. *duboisii*, African histoplasmosis, baboon (*Papio* spp.).

Histoplasmosis is a fungal infection caused by a pathogenic ascomycyte that is often found in a mycelial phase in soil contaminated with bird or bat guano.[1] Transmission can be via inhalation, ingestion or cutaneous inoculation through breaks in the epidermis.[2] *Histoplasma* can be divided into three subtypes of *Histoplasma capsulatum* var *capsulatum*, *duboisii*, and *farciminosum*, which are further separated into eight phylogeographic clades.[1] The clades are based on geographic location of the isolates because phylogenetic distinction between *Histoplasma* species is not considered useful for distinguishing between groups.[1] The eight clades are North America 1 (NAm-1), North America 2 (NAm-2), Eurasia, Australia, Netherlands, Africa, Latin America A (LAm-A), and Latin America B (LAm-B).[1,3,4]

African histoplasmosis is caused by *Histoplasma capsulatum* var. *duboisii* (*Hcd*) and causes granulomatous inflammation of the skin, bone, reproductive tissues, and oral cavity.[5] *Hcd* is endemic in countries of Western and Central Africa and Madagascar.[6] Cases of natural infection have been recognized primarily in African countries in both humans and baboons and in isolated case reports in humans with a travel history to Africa.[7–9]

The present report focuses on African histoplasmosis in a troop of baboons housed at a non-human primate facility in Texas, in which *Hcd* infection first occurred after the import of a group of red baboons (*Papio cynocephalus papio*) from Senegal.[2,5,10,11] The cases in baboons began approximately 18 months after the importation, and *Hcd* has been diagnosed in animals that had been born at the facility or housed at the facility for 10–12 years.[2] The purpose of this report is to determine the clade to which *Histoplasma* isolated from baboons housed at the primate facility belongs and compare it to the known phylogeographic clades.

Formalin-fixed, paraffin embedded (FFPE) tissues from 9 baboons from a research colony in Texas histologically diagnosed with *Hcd* were used in this study. Fifty micrometer curls from FFPE tissue blocks of testicle, penis, vulva, bone, tail, gingiva, and skin were used for DNA extraction and fungal amplification using ITS-2 region followed by sequencing, as previously described.[12] Briefly, DNA was extracted using the MO Bio BiOstic FFPE Tissue DNA Isolation kit (MO Bio, Carlsbad, CA, USA) according to the manufacturer's instructions. Amplification and sequencing of the ITS2 region (ITS3/4) was performed using primers ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′.[13] Additionally, amplification and sequencing for α-tubulin (Tub-1 5′ AGCTCCATTACAACAGCCAAT-3′ 5′CTGCCGAGGAGGAACAGTTA-3′) and ADP-ribosylation factor (Arf 5′CCATTGGTAAGTTCCTCGATTC-3′ 5′ACCGACGTCCCACACTGTAA-3′) was performed as previously described.[3]

Sequencing was performed by Eton BioScience, Inc. (San Diego, CA, USA). Sequence quality was analyzed using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA). Trimmed sequences were aligned to NCBI archived sequences using Basic Local Alignment Search Tool (BLAST). Sequences for ITS2, Tub-1 and Arf were edited, aligned, and concatenated using Molecular Evolutionary Genetic Analysis Software 6.0.6 (MEGA6) (http://www.megasoftware.net). Sequences for ITS2 (MG066693- MG066699), Tub-1 (MG100832-MG100840), and Arf (MG132212- MG132220) were deposited in GenBank. MLST sequences from 82 *H. capsulatum* strains representing the eight *H. capsulatum* clades were downloaded from the TreeBase database (http://www.treebase.org). A phylogenetic tree based on ITS2, a 278 bp region of the Arf gene, and a 470 bp region of the Tub-1 gene was constructed using the neighbor-joining method with 1000 bootstrap replicates in MEGA6 and was submitted to http://www.treebase.org.

The diagnosis of histoplasmosis was confirmed in seven cases by polymerase chain reaction (PCR) amplification using the ITS2 primer set. By concatenating ITS2, Tub-1, and Arf all nine samples identified as *Hcd* through BLAST analysis in GenBank. Using neighbor-joining analysis, all nine cases from baboons from the primate facility in Texas clustered together (Fig. 1) and demonstrated 88% gene homology within the Africa clade. The samples from baboons in Texas also clustered with a sample isolated from an individual in Senegal in 1957 (Fig. 1).

This report confirms the diagnosis of African histoplasmosis in a country outside of Africa and supports the imported baboons from Senegal as the origin for the index case that introduced *Hcd* to Texas. *Hcd* is now considered endemic within the facility with cases occurring sporadically over the last 30 years. Since the introduction, cases have been documented in baboons born at the facility who did not have contact with the original imported individuals; however, the mode of transmission has not been determined. Possible routes of transmission include ingestion of soil or contact with an infected individual.

An 18-month lag occurred between the introduction of infected red baboons (*Papio cynocephalus papio*) from Senegal and the development of clinical disease.[2] The time between infection and demonstration of clinical disease can be quite varied. Cases in people living in nonendemic regions have been reported up to 18- and even 40-year lag after presumed exposure during a stay in an endemic African country and the demonstration of clinical illness.[9,14] These cases presumably represent reactivation of latent infection.[14]

The majority of cases of histoplasmosis reported in the United States are caused by *Histoplasma capsulatum* var. *capsulatum*, which infects a wide variety of species and is predominantly within the NAm-1 and NAm-2 clades.[1,15] The primary clinical syndrome centers on the lungs secondary to inhalation of microconidia.[16] Approximately 90% of those infected will be subclinical or develop acute, flu-like pulmonary illness.[15,16] A chronic form manifests as cavitary or nodular pulmonary lesions and is associated with a history of smoking.[16] Immunosuppression can lead to the development of disseminated illness.[15] In contrast to *Histoplasma capsulatum* var. *capsulatum*, underlying immunosuppression is not typically a feature of reactivation of *Hcd*.[14] The baboons in this study were not considered immunosuppressed at the time of diagnosis.

In conclusion, this study highlights the diagnosis of African histoplasmosis outside of the historically accepted endemic regions and provides epidemiological evidence of the origin of the disease. Additionally, it demonstrates formalin-fixed paraffin embedded archived tissues are a valuable source of material for epidemiological and diagnostic studies.

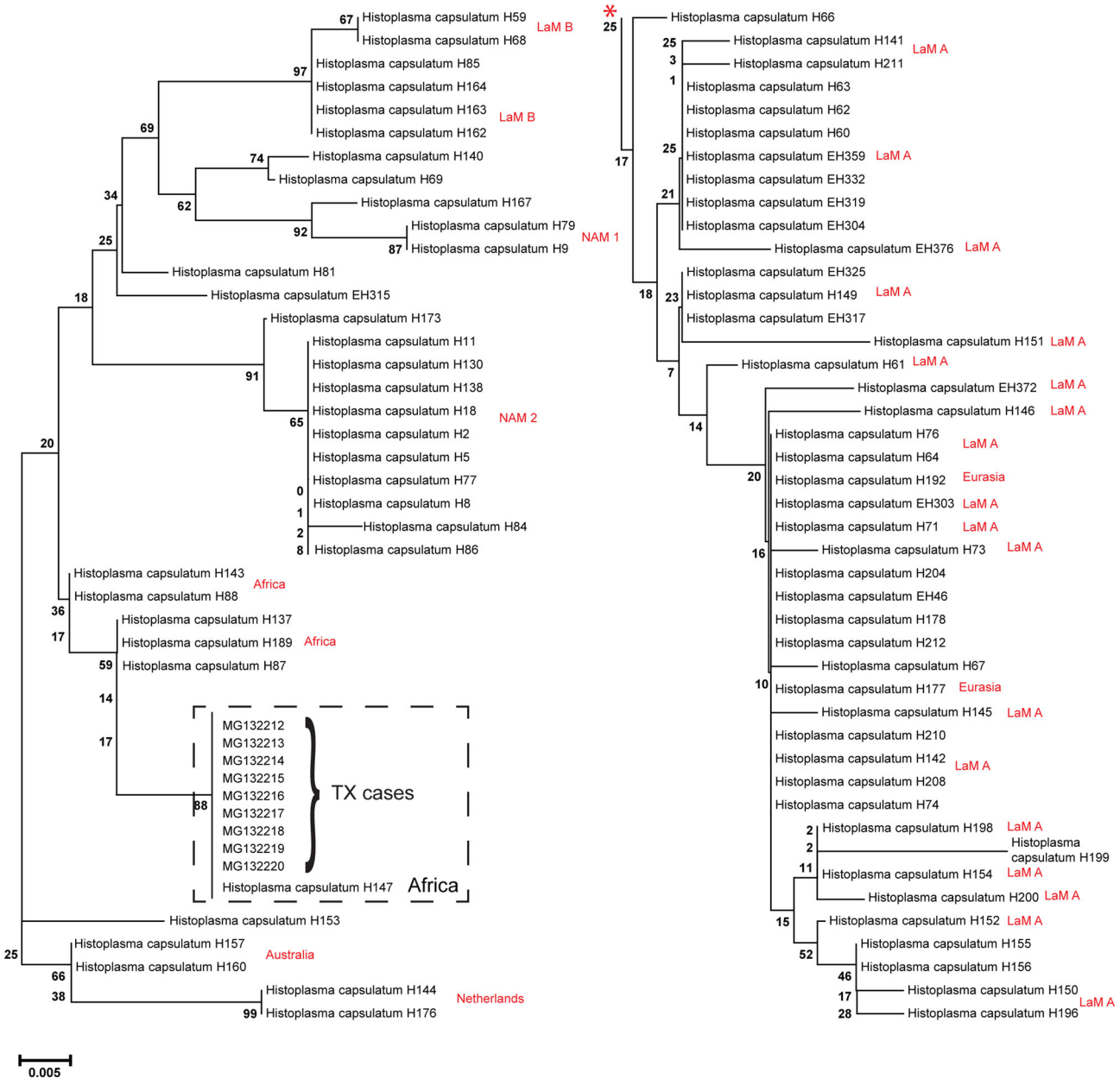

Figure 1. Neighbor-joining phylogenetic analysis of concatenated *Histoplasma* sequences from three gene loci; ITS2, arf, and Tub-1. *Histoplasma* sequences representing the eight geographic clades from http://www.treebase.org are included for comparison. LAm-A, Latin America group A clade; LAm-B, Latin America group B clade; NAm-1, North America 1 clade; NAm-2, North America 2 clade. The numbers in the dendrogram represent the bootstrap value after 1000 replicates. Scale bar = 0.005 amino acid substitutions per site. This Figure is reproduced in color in the online version of *Medical Mycology*.

Acknowledgments

This investigation was supported by Southwest National Primate Research Center [P51 RR013986] from the National Center for Research Resources and the National Institutes of Health, which are currently supported by the Office of Research Infrastructure Programs [P51 OD011133]. This investigation was conducted in facilities constructed with support from the Office of Research Infrastructure Programs (ORIP) of the National Institutes of Health [C06 RR015456, C06 RR014578].

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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