Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Invertebrate Pathology 88 (2005) 108–115
Journal of INVERTEBRATE PATHOLOGY
www.elsevier.com/locate/yjipa

Transmission of *Vairimorpha invictae* (Microsporidia: Burenellidae) infections between red imported fire ant (Hymenoptera: Formicidae) colonies

David H. Oi[a,*], Juan A. Briano[b], Steven M. Valles[a], David F. Williams[a]

[a] *USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608, USA*
[b] *USDA-ARS South American Biological Control Laboratory, Bolivar 1559 (1686), Hurlingham, Buenos Aires Province, Argentina*

Received 1 October 2004; accepted 19 November 2004
Available online 20 January 2005

Abstract

Red imported fire ant, *Solenopsis invicta*, colonies were successfully infected with the microsporidium *Vairimorpha invictae* by introducing live larvae, pupae, or dead adults from *V. invictae*-infected field colonies collected in Argentina. Introductions with 4th instar larvae or non-melanized pupae obtained from infected field colonies, resulted in infection of 40% of the inoculated colonies. Introductions of 4th instars or melanized pupae produced from colonies that were initially infected in the laboratory, resulted in infections of 83% of the colonies, thus perpetuating the infection in other colonies. Infection was detected in 2 of 6 colonies after introducing adult worker caste ants that had died with *V. invictae*. The average number of adults and the volume of immature ants per colony were significantly lower in the infected than in the control colonies. Infected colonies had 86% fewer adults per colony and 82% less immature ants than the controls. A portion of the 16S rRNA gene of the *V. invictae* identified from these studies was amplified, cloned, and sequenced; the 1251 nucleotide amplicon was 100% identical to the 16S rRNA gene sequence recorded previously in the GenBank database, thus verifying the species as *V. invictae*. This is the first report of the artificial transmission of this pathogen to uninfected ant colonies, and demonstration of its ability to hinder growth in individual colonies.
Published by Elsevier Inc.

Keywords: *Vairimorpha invictae*; Microsporidia; *Solenopsis invicta*; Fire ant; Pathogen; Transmission; Biological control

1. Introduction

Vairimorpha invictae is a microsporidium that was first described by Jouvenaz and Ellis (1986) infecting a colony of red imported fire ants, *Solenopsis invicta*, collected in the state of Matto Grosso, Brazil. *V. invictae* produces dimorphic spores, where large numbers of binucleate free spores occur in pupae and adult ants, and uninucleate octospores contained in sporophorous vesicles are present only in adults (Jouvenaz and Ellis, 1986). This contrasts with *Thelohania solenopsae* another microsporidium that infects fire ants, which has four reported spore types that are present in various ant developmental stages. Specifically, uninucleate octospores, or meiospores, contained in sporophorous vesicles are present in adults and pupae (Knell et al., 1977); binucleate, *Nosema*-like spores, or free spores, present primarily in adults but also reported in pupae and larvae (Knell et al., 1977; Sokolova et al., 2004); binucleate spores from pupae (Shapiro et al., 2003); and binucleate megaspores observed in adults and brood (Sokolova et al., 2004). A reported fifth spore, the macrospore, was recently considered to be abnormal octospores, or teratospores (Sokolova et al., 2004).

Surveys for pathogens of fire ants in South America indicated that *V. invictae* had a moderately low overall

* Corresponding author. Fax: +1 53 374 5818.
E-mail address: doi@gainesville.usda.ufl.edu (D.H. Oi).

0022-2011/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.jip.2004.11.006

prevalence, being found in 2.3% of 2528 colonies surveyed (Briano and Williams, 2002). However, at several *S. invicta* sites in Santa Fe Province, Argentina, *V. invictae* reached epizootic levels with 50% of the colonies infected (Briano and Williams, 2002). In a survey of black imported fire ants, *Solenopsis richteri*, and *Solenopsis quinquecuspis* in Buenos Aires Province, Argentina, *V. invictae* was detected in 1% of 1836 colonies compared to 8% for *T. solenopsae* (Briano et al., 1995). Preliminary observations of natural field infections of *V. invictae* in *S. invicta* suggested that this pathogen could reduce colony populations and had the potential to be an effective biological control agent (Briano et al., 2002). In addition, enhanced decline in colonies has been suggested in natural simultaneous infections of *V. invictae* and *T. solenopsae* (Briano, 2005). To further assess the potential of *V. invictae* as a biological control agent for introduction into the US, infections initiated in the laboratory would facilitate host specificity and colony impact testing. Previous attempts to transmit this pathogen to small (10,000 adult ants), uninfected *S. invicta* colonies by providing boiled chicken egg yolk wetted with spore suspensions or by introducing brood from infected colonies were unsuccessful (Jouvenaz and Ellis, 1986). Nevertheless, our objectives were to infect *S. invicta* colonies with *V. invictae* under laboratory conditions and to document the impact of infection on colony growth.

2. Materials and methods

Vairimorpha invictae-infected *S. invicta* colonies were collected near San Javier, Santa Fe province, Argentina in 2003 and transported to quarantine facilities at the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida. Infected colonies were identified by examining aqueous extracts of macerated groups of adults for spores of *V. invictae* and *T. solenopsae* using phase contrast microscopy (Briano and Williams, 2002) and comparing morphological features of free spores and octospores (meiospores) described by Jouvenaz and Ellis (1986). Voucher specimens of *V. invictae* were deposited in the collection of the senior author in the form of giemsa-stained slides and alcohol preserved ants from infected colonies. Additional slides were added to the collection of Dr. James Becnel at the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida, USA.

2.1. Molecular and morphological verification of microsporidian species

To further verify the identity of *V. invictae*, the 16S rRNA gene was amplified, cloned, sequenced, and compared with sequences in the GenBank database. DNA was extracted from *V. invictae*-infected *S. invicta* by the method of Valles et al. (2002). The *S. invicta* was obtained from a colony that was infected in the study described in Sections 2.3 and 3.3. Oligonucleotide primers were designed to the *Vairimorpha* sp. 16S rRNA gene sequence (Accession No. AF031539) reported previously by Moser et al. (1998). PCR was carried out with forward (5′-TCAGAGATTAAGCCATGCAAGCCAG) and reverse (5′-TGTATCCAATCTACAAGCACA GATTCGTC) oligonucleotide primers under the following temperature regime: 94 °C for 2 min, then 35 cycles at 94 °C for 15 s, 57 °C for 15 s, and 68 °C for 2 min, followed by a polishing step of 68 °C for 5 min. The 1251 nucleotide amplicon was ligated into the pCR-4 vector and transformed into TOP TEN competent *Escherichia coli* (In Vitrogen, Carlsbad, CA). Four clones were randomly selected and sequenced by the Interdisciplinary Center for Biotechnology Research, University of Florida. Contiguous nucleotide sequences were analyzed by BLAST (Altschul et al., 1997) and sequence comparisons were made using the Vector NTI Suite ver. 7.0 (Informax, Bethesda, MD).

Wet mounts were prepared from groups of approximately 10 macerated adult workers from each of two colonies that were inoculated with *V. invictae* infected brood (Sections 2.2 and 2.3). Worker samples were collected at least 28 weeks after inoculation. The length and width of free spores and meiospores were measured with an image-splitting micrometer (Vickers A.E.I., Vickers Instruments, Malden, MA) (Undeen, 1997).

2.2. Inoculations with larvae or non-melanized pupae from field colonies

Inocula consisted of brood collected from a colony infected with *V. invictae*. Infection rates of the inocula were determined to be 100% based on both individual wet mounts of 10 pupae and 10 giemsa-stained larval smears (Undeen, 1997) that were examined by microscopy for *V. invictae* spores or vegetative stages, respectively. Incipient colonies used for inoculations and controls were reared (Banks et al., 1981; Oi and Williams, 2003) from *S. invicta* queens, that were collected within a day of nuptial flights (newly mated queens), in Alachua and Orange counties Florida. Colonies most likely possessed the monogyne genotype, because queens with this genotype have the most capacity to disperse and independently found colonies (DeHeer et al., 1999). Colonies were inoculated when they were 5–6 weeks old and contained approximately 400 pieces of brood (1 ml), 30 workers, and 1 queen. Because removing ants from such small incipient colonies could impair the colonies, and because *V. invictae* is not known to occur in the US (Beckham et al., 1982; Broome, 1974; Jouvenaz, 1986; Jouvenaz et al., 1977; Pereira et al., 2002; D.H.O. unpublished data), colonies were not examined for infection