Journal of the American Mosquito Control Association, 9(3):269–274, 1993

MOSQUITO HOST RANGE AND SPECIFICITY OF *EDHAZARDIA AEDIS* (MICROSPORA: CULICOSPORIDAE)

JAMES J. BECNEL AND MARGARET A. JOHNSON

U.S. Department of Agriculture, Agriculture Research Service, Medical and Veterinary Entomology Research Laboratory, Gainesville, FL 32604

ABSTRACT. *Edhazardia aedis* was transmitted horizontally to its natural host, *Aedes aegypti*, and to 6 alternate hosts: *Ae. albopictus, Ae. triseriatus, Ae. taeniorhynchus, Anopheles quadrimaculatus, Orthopodomyia signifera* and *Toxorhynchites rutilus rutilus*. The microsporidium produced both binucleate and uninucleate spores in all susceptible hosts. Transovarial transmission, however, was only successful in *Ae. aegypti*. Therefore, while *E. aedis* can infect a variety of mosquito species from diverse genera, it is specific for its natural host, *Ae. aegypti*. Five other mosquito species were not susceptible to *E. aedis*.

INTRODUCTION

Edhazardia aedis (Kudo) was originally observed and described from *Aedes aegypti* (Linn.) in Puerto Rico (Kudo 1930) and reisolated in Thailand (Hembree 1979). This microsporidium has a complex life cycle termed the "parental-host filial-host alternation pathway" (Becnel et al. 1989); part of this pathway involves transovarial transmission from infected adult females to progeny via a binucleate spore (Hembree and Ryan 1982). Uninucleate, pyriform spores are formed in the fat body of these progeny, and this process is normally fatal. Spores released from these dead individuals into the aquatic environment are infectious *per os* to *Ae. aegypti* larvae yielding infected adults to complete development. Because of these and other life cycle features, *E. aedis* has potential as a biocontrol agent for container-inhabiting mosquitoes (Becnel 1990).

Hembree (1982) reported that *Ae. aegypti* and *Ae. taeniorhynchus* (Wied.) were susceptible to *E. aedis*; *Culex quinquefasciatus* Say and *Anopheles stephensi* Liston were not susceptible. *Aedes taeniorhynchus* was found to be "almost as infectious" to *E. aedis* as *Ae. aegypti*, but no details were given on other host–parasite interactions. The purpose of the present study was to further define the mosquito host range of *E. aedis* and to examine the interactions of the parasite with the susceptible alternate hosts.

MATERIALS AND METHODS

The isolate of *E. aedis* used originated from Thailand and has been maintained since 1981 in laboratory colonies of *Ae. aegypti* according to the methods of Hembree and Ryan (1982). Larvae from infected eggs were reared at 27°C for 7–9 days, and patently infected larvae were isolated for transmission experiments. A portion of these larvae were triturated in a glass tissue grinder, and large particulate matter was removed from this extract by forcing it through cotton in a syringe. The inoculum was washed and centrifuged 3 times according to the protocol of Undeen and Maddox (1973), held at 20°C and used within 24 h. Spore concentrations were determined with a hemocytometer prior to feeding.

Twelve mosquito species representing 7 genera were bioassayed for susceptibility to *E. aedis* (Table 1). Mosquitoes for bioassays either were obtained from existing laboratory colonies or were field-collected from the environs of Gainesville, Florida.

Second instar mosquito larvae (~48 h old) were exposed in groups of 100 in 100 ml of a spore suspension for 24 h. A range of doses between 1×10^2 and 1×10^5 spore/larva was used to calculate estimates of the IC_{50} and LC_{50} dosages. For species that were not susceptible to *E. aedis* in this range of doses, a maximum challenge of 1×10^6 spores/larva was administered. All treatment groups were then transferred to pans with 500 ml of water and fed according to standard rearing protocols. Second instar larvae (72 h old) of the predacious mosquito, *Toxorhynchites rutilus rutilus* (Coq.), were isolated and fed one infected 4th instar *Ae. aegypti* larva (one larva $= 3 \times 10^5$ spores) and healthy larvae thereafter. Second instar larvae of *Ae. aegypti* were exposed with each test to verify spore viability. Control groups were used in all tests and handled in a similar manner but without the addition of spores.

Individuals from the exposed groups were sampled approximately 1 h post-exposure, and their gut contents were examined with phase microscopy for the presence and condition of spores. Mortality was recorded when pupation began, and a sample of the surviving individuals was smeared on microscope slides and stained with a Giemsa-stain solution; percentage of infection was determined microscopically. The IC_{50} and LC_{50} dosages were calculated when possible by probit analysis.

Table 1. Mosquito host range of *Edhazardia aedis*.

Mosquito host	Source	No. exposed (replications)	IC_{50}[1]	95% confidence interval	LC_{50}[1]	95% confidence interval	Transovarial transmission	
							No. exam.	No. pos.
Aedes aegypti	C[2]	10,000 (10)	39	29–49	1,270	560–2,400	22	22
Aedes albopictus	C	300 (3)	84	68–126	3,800	975–29,423	17	0
Aedes taeniorhynchus	C	300 (3)	3,080	1,758–5,145	29,600	18,675–52,962	15	0
Aedes triseriatus	C	300 (3)	29.7	2–100	2,280	1,600–3,500	30	0
Anopheles quadrimaculatus	C	400 (4)	2.5	—	58,500	16,000–200,000	20	0
Anopheles albimanus	C	300 (3)	0	—	—	—	N/A	N/A
Culex nigripalpus	FC[3]	300 (1)	0	—	—	—	N/A	N/A
Culex quinquefasciatus	C	400 (4)	0	—	—	—	N/A	N/A
Culiseta melanura	FC	250 (2)	0	—	—	—	N/A	N/A
Orthopodomyia signifera	FC	50 (1)	pos.	—	—	—	N/A	N/A
Uranotaenia sapphirina	FC	22 (2)	0	—	—	—	N/A	N/A
Toxorhynchites rutilus rutilus	C	74 (2)	pos.	—	—	—	8	0

[1] Spores per individual.
[2] Colonized.
[3] Field-collected.

Two methods were required to determine if the parasite could complete its life cycle in an alternate mosquito host. One was to examine infected female adults (after oviposition) for the presence of binucleate spores. If binucleate spores were observed, progeny from these infected females were examined for the presence of infection and or pyriform spores. The other method was to hold infected individuals with extended development (either larvae, pupae or adults) and examine them for spores (binucleate and pyriform spores) at various times post-exposure. If pyriform spores were produced in the alternate host, these were bioassayed in *Ae. aegypti* larvae to determine viability.

RESULTS

Uninucleate spores of *E. aedis* were observed among the gut contents of individuals from each species of mosquito tested. Ungerminated spores were easily recognized by their highly refractile appearance as demonstrated in the natural host, *Ae. aegypti* (Fig. 1), and in *Cx. quinquefasciatus* (Fig. 2). Spores of *E. aedis* readily germinated in all mosquitoes tested and were identified by their dark color and clearly defined spore wall (Figs. 1 and 2). In *Ae. aegypti,* the everted polar tube penetrated the peritrophic membrane (Fig. 1), but this was not observed in *Cx. quinquefasciatus* (Fig. 2).

Edhazardia aedis was transmitted to 7 mosquito species representing 4 genera: *Ae. aegypti, Ae. albopictus* (Skuse), *Ae. triseriatus* (Say), *Ae. taeniorhynchus, An. quadrimaculatus* Say, *Orthopodomyia signifera* (Coq.) and *Tx. rutilus rutilus.* Estimates for the IC_{50} and LC_{50} are given in Table 1. There was no evidence of infection in the other mosquito species tested.

Developmental stages of *E. aedis* in the alternate mosquito hosts appeared as previously described (Becnel et al. 1989) beginning with uninucleate stages (gamonts and gametes), followed by plasmogamy and nuclear association to form diplokaryotic meronts. These stages underwent sporulation (sporogony plus sporogenesis) to form binucleate spores. Binucleate spores were found in larvae, pupae and adults of each mosquito host that became infected with *E. aedis* (Figs. 3 and 4). In the alternate hosts, these spores were determined to be functionally mature by their normal germination and eversion of the polar tube (Fig. 4).

Edhazardia aedis was transmitted transovarially and therefore completed its life cycle in all families of *Ae. aegypti* examined ($n = 22$). No infections were found in the F_1 progeny of individual families from infected females (positive for binucleate spores) of *Ae. albopictus* ($n = 17$), *Ae. triseriatus* ($n = 30$), *Ae. taeniorhynchus* ($n = 15$), *An. quadrimaculatus* ($n = 20$) or *Tx. rutilus rutilus* ($n = 8$). Transovarial transmission in *Or. signifera* could not be determined.

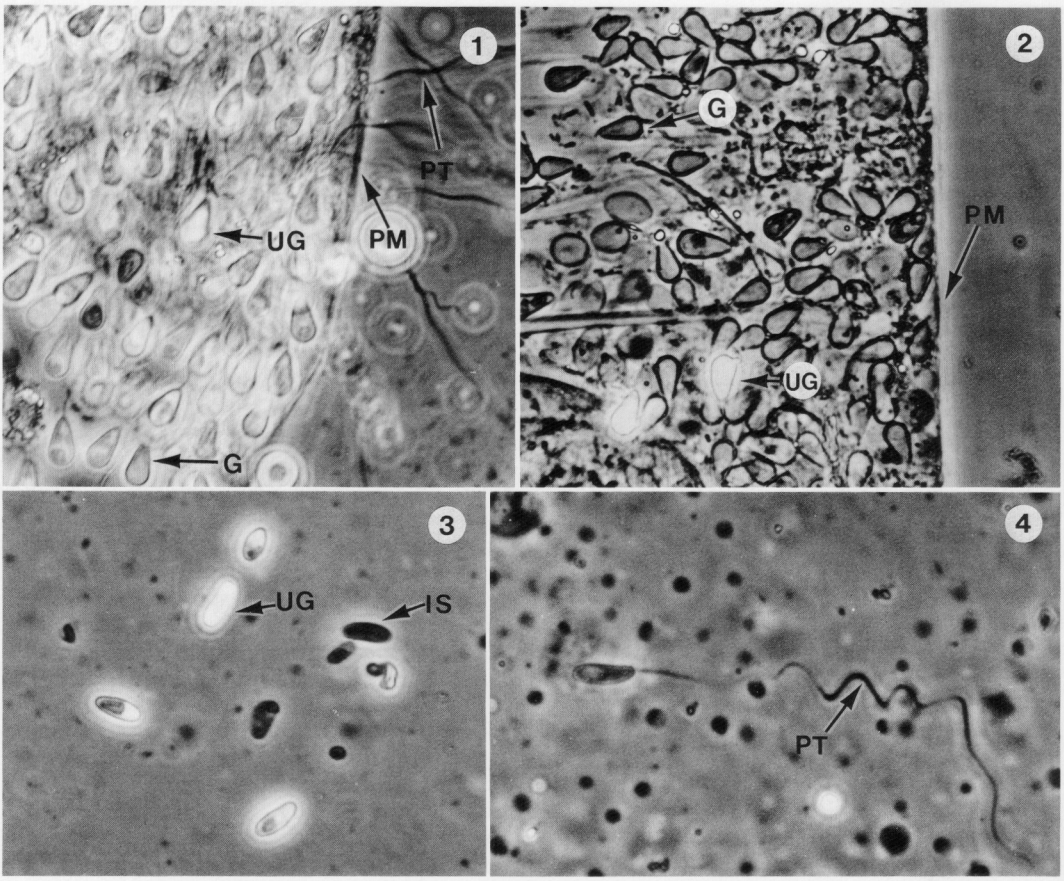
Uninucleate, pyriform spores were found in horizontally (orally) infected *Ae. aegypti, Ae. albopictus, Ae. triseriatus, Ae. taeniorhynchus* and *Tx. rutilus rutilus* with extended development (>12 days). Regardless of the host in which they were formed, these uninucleate spores were infectious *per os* to *Ae. aegypti* larvae in which they initiated the normal developmental sequence leading to the production of binucleate spores.

DISCUSSION

Edhazardia aedis was transmitted horizontally to its natural host, *Ae. aegypti,* and 6 alternate hosts: *Ae. albopictus, Ae. triseriatus, Ae. taeniorhynchus, An. quadrimaculatus, Or. signifera* and *Tx. rutilus rutilus.* The dosage of *E. aedis* which produced mortality when exposed to 2nd instar *Ae. aegypti* (LC_{50} = 1,270 spores/larva) was approximately 30 times the dose required to infect (IC_{50} = 39 spores/larva). These dosages are similar to those reported by Hembree (1982). The estimated IC_{50} and LC_{50} for the alternate hosts varied greatly with either large or incalculable confidence intervals (Table 1), which indicated that individual responses are not predictable for the unnatural hosts. *Anopheles quadrimaculatus* was the most susceptible species to *E. aedis* (IC_{50} = 2.5) but also required the highest dose for mortality (LC_{50} = 58,500). The other species responded similarly to *Ae. aegypti* except for *Ae. taeniorhynchus,* which was considerably less susceptible. This differed from Hembree (1982) who found similar IC_{50}s for 24-h-old *Ae. aegypti* and *Ae. taeniorhynchus* larvae exposed to *E. aedis.* This discrepancy could be due to the age of larvae used for the comparison or to strain differences between the mosquito species.

Normal vegetative growth and development occurred in all of the susceptible species, and *E. aedis* did sporulate to form binucleate spores in larvae and adults. In its natural host, *Ae. aegypti,* binucleate spores were formed in oenocytes that invaded the ovaries (Becnel et al. 1989) and consistently resulted in transovarial transmission to larval progeny (Table 1). However, *E. aedis* was not transovarially transmitted to the filial generations in any of the susceptible, alternate mosquito hosts examined, even though mature binucleate spores were present and presumably germinated in these species.

In what was described as "deviations from the parental-host filial-host alternation," Becnel et al. (1989) described 2 alternate pathways by which *E. aedis* completes its developmental cycle in *Ae.*



Figs. 1–4. Spores of *Edhazardia aedis*. Fig. 1. Uninucleate spores within the peritrophic membrane of *Aedes aegypti*. Fig. 2. Uninucleate spores within the peritrophic membrane of *Culex quinquefasciatus*. Fig. 3. Binucleate spores from adult *Ae. triseriatus*. Fig. 4. Germinating binucleate spore from adult *Ae. albopictus*. G, germinated spore; UG, ungerminated spore; PM, peritrophic membrane; PT, polar tube; IS, immature spore. All fresh preparations and 1,000×.

aegypti. One of these involves individuals infected *per os* as larvae whose longevity extended up to 2 wk. In these individuals, the entire developmental cycle of *E. aedis* was often completed by producing binucleate spores which presumably germinated (autoinfection) to initiate the sequences leading to the production of uninucleate spores. This alternate pathway may serve a supportive role for parasite maintenance within *Ae. aegypti* populations under certain conditions. In the current study, a similar pathway was found in each of the alternate mosquito hosts in which *E. aedis* completed its developmental cycle in those infected individuals with an extended development time. This ability of *E. aedis* to complete its developmental cycle in alternate hosts might also serve a supportive role to the primary parental-host filial-host pathway in *Ae. aegypti*. While the species assemblage used in this study is not usually associated with the natural host, it demonstrates that alternate hosts in the natural system may provide reservoirs for maintaining and amplifying the parasite when *Ae. aegypti* numbers are low. Maintenance of *E. aedis* within living hosts seems necessary, especially due to the fragile nature of the uninucleate spores, which would not be expected to persist for long periods once released into the environment (Undeen and Becnel 1992).

Andreadis (1989) demonstrated that 4 alternate mosquito hosts were susceptible to oral infection with *Amblyospora connecticus* Andreadis from the natural host *Ae. cantator* (Coq.); however, binucleate spores were produced only in female *Ae. epactius* Dyar and Knab. These binucleate spores did not invade or germinate in ovarian tissues, and *A. connecticus* could not complete its life cycle via transovarial transmis-

sion. Andreadis concluded that *A. connecticus* is specific for *Ae. cantator* and that host specificity operates at 3 different levels: larval infectivity, sporulation and spore germination with ovarian infection. While the host specificity of *E. aedis* was clearly operational at the larval infectivity level, specificity was not related to the sporulation or germination of binucleate spores. Both binucleate and uninucleate spores of *E. aedis* were produced in each of the alternate mosquito hosts tested, and germination of binucleate spores seems likely as this is assumed to be a prerequisite for the production of uninucleate spores (Becnel et al. 1989). While *E. aedis* and *A. connecticus* are similar in that transovarial transmission is not successful in alternate hosts, *E. aedis* differs in the ability to complete its developmental cycle in alternate hosts in the absence of transovarial transmission. Therefore, host specificity of *E. aedis* cannot be defined by developmental parameters, such as sporulation, germination or completion of the developmental cycle, but rather by the successful transovarial transmission of the parasite to the next generation.

Uninucleate spores germinated in the guts of all experimental mosquito larvae, and germination was not correlated with the susceptibility of the host to *E. aedis*. Uninucleate spores of *E. aedis* also germinated in the guts of a variety of nontarget aquatic organisms but did not produce infections (Becnel 1992). Based on previous studies with *Nosema algerae* Vavra and Undeen, Undeen (1976) speculated that the lack of detectable infections was due to a gut barrier preventing the infective germ from entering the hosts rather than some physiological incompatibility preventing parasite development. Our observations of the polar tube penetrating the peritrophic membrane of a susceptible host, *Ae. aegypti* (Fig. 1), and not penetrating the membrane of a refractory host, *Cx. quinquefasciatus* (Fig. 2), support this hypothesis.

The environmental safety of a microbial control agent is evaluated, in part, on knowledge of its host range. Emphasis has been placed on selecting candidates with both a wide host range for target pests and a minimal risk to nontarget organisms (Brooks 1988). For microsporidia with complex host–parasite relationships, there is a clear difference between the host range of a particular species and its specificity. This study has demonstrated that *E. aedis* can infect a variety of mosquito species representing diverse genera and, therefore, has a broad host range. However, *E. aedis* is specific for its natural host, *Ae. aegypti*, based on its ability to complete its life cycle via transovarial transmission, which does not occur in the alternate mosquito hosts tested. The criteria needed to establish host limits of certain microsporidia must therefore consider the differences between host range and host specificity. This has important implications for the taxonomic determination of specific microsporidia as well as identifying suitable target hosts for biological control projects.

Because of its specificity for *Ae. aegypti*, *E. aedis* has its greatest potential as a biocontrol agent for its natural host, *Ae. aegypti*. Since both horizontal and transovarial transmission are necessary for maintenance of parasites like *E. aedis* in mosquito populations (Andreadis and Hall 1979), it is unlikely that *E. aedis* could persist in an alternate mosquito host without a functional transovarial transmission sequence.

ACKNOWLEDGMENTS

Our sincere appreciation goes to Genie White for her excellent technical assistance with the statistical analyses. We also thank T. G. Andreadis, T. Fukuda and A. H. Undeen for their helpful suggestions and comments on an earlier draft of the manuscript.

REFERENCES CITED

Andreadis, T. G. 1989. Host specificity of *Amblyospora connecticus*, a polymorphic microsporidian parasite of *Aedes cantator*. J. Med. Entomol. 26:140–145.

Andreadis, T. G. and D. W. Hall. 1979. Development, ultrastructure, and mode of transmission of *Amblyospora* sp. (Microspora) in the mosquito. J. Protozool. 26:444–452.

Becnel, J. J. 1990. *Edhazardia aedis* (Microsporida: Amblyosporidae) as a biocontrol agent of *Aedes aegypti* (Diptera: Culicidae), pp. 56–60. Proc. Vth Int. Colloq. Invertebr. Pathol. Microb. Control, Adelaide, Australia.

Becnel, J. J. 1992. Safety of *Edhazardia aedis* (Microspora: Amblyosporidae) for nontarget aquatic organisms. J. Am. Mosq. Control Assoc. 8:256–260.

Becnel, J. J., V. Sprague, T. Fukuda and E. I. Hazard. 1989. Development of *Edhazardia aedis* (Kudo, 1930) n.g., n. comb. (Microsporida: Amblyosporidae) in the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). J. Protozool. 36:119–130.

Brooks, W. M. 1988. Entomogenous Protozoa, pp. 1–149. *In:* C. M. Ignoffo and N. B. Mandava (eds.). CRC handbook of natural pesticides. Volume V. Microbial insecticides. Part A. Entomogenous Protozoa and Fungi. CRC Press, Inc., Boca Raton, FL.

Hembree, S. C. 1979. Preliminary report of some mosquito pathogens from Thailand. Mosq. News 39: 575–582.

Hembree, S. C. 1982. Dose–response studies of a new species of *per os* and vertically transmittable microsporidian pathogen of *Aedes aegypti* from Thailand. Mosq. News 42:55–61.

Hembree, S. C. and J. R. Ryan. 1982. Observations

on the vertical transmission of a new microsporidian pathogen of *Aedes aegypti* from Thailand. Mosq. News 42:49–54.

Kudo, R. 1930. Studies on microsporidia parasitic in mosquitoes. VIII. On a microsporidian, *Nosema aedis* nov. spec., parasitic in a larva of *Aedes aegypti* of Puerto Rico. Arch. Protistenkd. 69:23–28.

Undeen, A. H. 1976. *In vivo* germination and host specificity of *Nosema algerae* in mosquitoes. J. Invertebr. Pathol. 27:343–347.

Undeen, A. H. and J. J. Becnel. 1992. Longevity and germination of *Edhazardia aedis* (Microspora: Amblyosporidae). Biocontrol Sci. Technol. 2:247–256.

Undeen, A. H. and J. V. Maddox. 1973. The infection of nonmosquito hosts by injection with spores of the microsporidan *Nosema algerae*. J. Invertebr. Pathol. 22:258–265.