FECUNDITY AND LONGEVITY OF *ANOPHELES ALBIMANUS* EXPOSED AT EACH LARVAL INSTAR TO SPORES OF *NOSEMA ALGERAE*[1]

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ABSTRACT. Laboratory tests were made to compare infection rates, reproductive capacity, and longevity of *Anopheles albimanus* Wiedemann when infections of *Nosema algerae* Vavra and Undeen were induced in each of the different larval instars. Infection rates of 100% were obtained in tests with 1st and 2nd instar larvae, and 88 and 92%, respectively, were obtained in tests with 3rd and 4th instar larvae. Infections of *N. algerae* sharply reduced the reproductive capacity and longevity of *An. albimanus* regardless of the instar that became infected, but the reductions were greatest when 1st and 2nd instars were exposed. The tests indicate that the introduction of *N. algerae* into natural populations of *An. albimanus* consisting of mixed larval instars could significantly reduce the number and fertility of eggs, and thus result in an average reduction of ca. 54% of the F_1 progeny from those females that become infected.

INTRODUCTION

Nosema algerae (Microsporida, Nosematidae Vavra and Undeen (=*Nosema stegomyiae* of Anthony et al. 1972) has been studied as a pathogen of mosquitoes at the Insects Affecting Man and Animals Research Laboratory, ARS, USDA, Gainesville, FL, since 1966. The results of these investigations have shown that *N. algerae* is highly pathogenic to certain anophelines and that the pathogen may have a potential use in a pest management program for these mosquitoes (Hazard 1970, Savage and Lowe 1970, Hazard and Lofgren 1971). Epizootics involving several species of colonized anophelines have been described (Canning and Hulls 1970 and Hazard 1970), and it is apparent that the disease seriously restricts the use of such colonies for virtually any investigative purpose. Larval mortality may be high, and adults that emerge show a marked reduction in reproductive capacity and in longevity. Laboratory tests (Anthony et al. 1972) have shown that continuous exposure of all larval instars of *Anopheles albimanus* Wiedemann to *N. algerae* caused infections that reduced the LT-90 of the resulting females by 50%. We therefore proposed a hypothetical population model to illustrate the probable effect of *N. algerae* on adult *An. albimanus* that are vectors of malaria. The model showed that an infection of *N. algerae* that reduced the LT-90 of females by 50% would reduce the number of potential vectors of malaria by 85–97%.

Recent tests in Panama (unpublished data) have demonstrated that *N. algerae* can be introduced into natural breeding areas of *An. albimanus* and significant infection rates can be obtained in the native larval populations. In these tests, spores were applied to natural habitats where all larval instars of *An. albimanus* were present. Laboratory observations (unpublished data) indicate that *N. algerae* usually requires about 5 days for sporulation, thus the massive infections seen in late instar larvae collected from the treated plots in the Panama field study were probably induced when the larvae were in the 1st or 2nd instars. Laboratory tests (unpublished data) have shown that 3rd and 4th instar larvae of *An. albimanus* became infected with *N. algerae*, but spores were not appar-

[1] The research reported in this manuscript was conducted in part with contract funds transferred from the Medical Research and Development Command, Office of the Surgeon General, U. S. Army.

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ent until the adults were several days old. The effect of the disease, if any, on these adult mosquitoes is unknown.

To determine the probable effect of *N. algerae* on populations consisting of all larval instars, laboratory tests were made to compare infection rates, reproductive capacity, and longevity of *An. albimanus* when the infections were induced in each of the larval instars.

MATERIALS AND METHODS

Two replicate tests were conducted as follows.

REARING PROCEDURES. Newly hatched larvae of the Apastepeque strain of *An. albimanus* were counted into white enamel pans (18 × 29 × 4.5 cm) containing 500 ml of well water. The water was infused with 20 ml of an aqueous suspension containing 1% of a mixture of brewer's yeast and hog chow supplement (2:1). The pans were held in the laboratory at 29.0±0.5°C. Four days later and each day thereafter until the larvae pupated, each pan received 20 ml of a 1% suspension containing a liver-hog chow mixture (1:1). Pupae were removed from the pans daily, placed in small waxed paper cups, and then held for emergence in aluminum C-frame cages covered with tube gauze (Savage and Lowe 1971). Cotton pads soaked with 10% sugar solution were provided.

INFECTION PROCEDURES. The El Salvador strain of *N. algerae* was used to infect each of the larval instars of *An. albimanus*. This strain was isolated from mosquitoes collected on the west coast of El Salvador in January 1971 and has since been maintained in the laboratory by periodic passage through *An. albimanus* and *Heliothis zea* (Boddie). Spores for the infection tests were obtained from 7 to 10-day-old adult mosquitoes, cleaned by the triangulation method (Cole 1970), and used within 30 days of the date of harvest.

To infect the larvae, 20 pans, each containing 150 larvae, were used for each of the 2 replicates (4 pans for each instar to be infected and 4 untreated control pans). Spores of *N. algerae* were added to the pans containing the newly-hatched larvae immediately after they were set up, and spores were administered to the other pans when it was apparent that 80% or more of the larvae had molted to the 2nd, 3rd, or 4th instars. All pans were treated at a rate of 5×10^4 spores/ml of water (3.4×10^4 spores/cm^2, a total of 2.5×10^7 spores/pan). Previous tests (Anthony et al. 1972) indicated that continuous exposure to this dosage would produce 95–100% infection with low larval mortality among newly-hatched, 1st instars.

TEST AND EVALUATION PROCEDURES. Pupation and emergence records were maintained on each pan to determine variations in rearing efficiency and mortality that might be attributed to the *Nosema* treatments. On the 3rd day after emergence, all mosquitoes were offered blood meals from guinea pigs, and on the following day individual, gravid females were isolated in plastic tubes (2.5 × 6.0 cm) lined inside with a strip of filter paper. To induce oviposition, 3-5 ml of water were added to each tube. Each female mosquito was assigned a number within its respective test group so that records could be maintained on an individual basis. After oviposition, the females were transferred to waxed paper cups covered with nylon netting and supplied with cotton pads saturated with 10% sugar solution. Additional blood meals were offered 2–4 days after each oviposition, and the females were again isolated in tubes. Eggs were counted, and percentage hatch was determined 72–96 hr after oviposition. Unhatched eggs from 3 to 5 females in each test group were examined for infections by phase microscopy at each gonotrophic cycle. Mortality of the females was recorded daily throughout the test period. Status of infection was determined by making Giemsa stained smears from each female after death. No data for uninfected specimens except for the controls were considered in our analysis of the results. Also, data were discarded for those females that (1) failed to oviposit on the 1st gonotrophic cycle, (2) produced sterile eggs on the 1st gonotrophic cycle, or (3)

escaped during transfer manipulations. Thus data were kept only for those individual females for which there was a complete record of oviposition until death.

RESULTS AND DISCUSSION

The number of gonotrophic cycles, number of eggs laid, and percentage hatch for each of the 2 replicates were subjected to multiple regression analyses. Comparisons of these analyses showed that there were no statistically significant differences between the replicates; therefore, the data reported herein are averages based on the total number of female mosquitoes used in both tests. The daily mortality data were subjected to probit analysis to compute LT-50 and LT-90 values.

REARING EFFICIENCY. At least 90% of the *An. albimanus* in all treatment groups and controls emerged as adults except for those exposed to *N. algerae* spores as newly-hatched, 1st instar larvae. In the 1st replicate there was 14% mortality of immature stages in this group; however, in the 2nd replicate there was only 9%. This order of mortality was within the expected range for 1st-instar larvae exposed to *N. algerae* at this dosage.

INFECTION RATES OF *N. algerae*. The average infection rates for adults in the 2 replicates were as follows: 1st and 2nd instars exposed—100%; 3rd instars exposed—88%; 4th instars exposed—92%; and control —0%. Thus the later instars of *An. albimanus* were nearly as susceptible to infection by *N. algerae* as the earlier instars.

EFFECTS OF INFECTION ON FECUNDITY. Infection by *N. algerae* markedly reduced the reproductive capacity of *An. albimanus* regardless of the larval instar that became infected (Table 1). Although the greatest effects were seen in those exposed as 1st instars, the fecundity of all females exposed as larvae was significantly (0.01% level) reduced when compared with the controls. There were no significant differences between those exposed as 1st and 2nd or between those exposed as 3rd and 4th instar larvae in regard to the number of gonotrophic cycles, eggs laid, or percentage hatch; however, the differences between those exposed as 2nd and 3rd instars were highly significant. This may be explained, at least in part, by the timing of exposures to spores to coincide with the larval molt. Spores were administered to the groups as follows: First instar—day 1 immediately after set up; 2nd instar—late day 2; 3rd instar—day 5; 4th instar—late day 6 (2nd replicate) and early day 7 (1st replicate). Thus, nearly 3 full days elapsed between exposures of the 2nd and 3rd instar groups. If a given dosage will have a specific pathogenic effect on fecundity or longevity when the infection reaches a certain level, then the attainment of this level is primarily a function of time, and not of

Table 1. Effects of *Nosema algerae* on the fecundity of *Anopheles albimanus* when infections were induced in different larval instars.

Parameters Evaluated (avg./♀)	Instars Infected				Uninfected controls
	1st	2nd	3rd	4th	
Total Females Tested	85	91	83	77	74
Gonotrophic cycles	1.8	1.9*	2.4	2.4	3.9
Eggs Laid	190.7	224.7	297.0	304.2	482.4
Eggs Hatched	154.3	180.2	244.4	240.6	445.1
Reduction in Eggs Hatched (%) Compared to Controls	66.0	60.0	45.0	46.0	

* No statistical differences at the 0.01% level between figures connected by the same line.

Table 2. Cumulative percentage mortality of *Anopheles albimanus* females after each gonotrophic cycle when infections of *Nosema algerae* were induced in different larval instars.

Gonotrophic cycle	% Mortality of Groups Exposed				
	1st instar	2nd instar	3rd instar	4th instar	Controls
1	36	27	10	6	11
2	72	86	55	53	21
3	97	100*	89	88	42
4	100*		96	97	62
5			100*	100*	78
6					92

* Remaining mosquitoes died before completing indicated gonotrophic cycle.

the instar infected. The fecundity data (Table 1) and the longevity data (Tables 2 and 3) support this proposition which suggests that at the dosage of spores used in these tests, infections of *N. algerae* developed at a similar rate regardless of the instar that became infected.

EFFECT OF INFECTION ON LONGEVITY. Table 2 gives the cumulative mortality of each group after each gonotrophic cycle. In both replicates, high rates of mortality were evident among the females exposed as 2nd instar larvae within 24 hr after the mosquitoes were offered the 2nd blood meal. We have no explanation for this early mortality since there was no evidence of significant change in the laboratory environment, and similar mortalities did not occur in other groups that were given blood meals from guinea pigs on the same day. However, examinations of Giemsa stained smears made from these dead mosquitoes showed that all were heavily infected with *N. algerae*. Throughout the studies we observed that many of the infected mosquitoes died soon after consuming blood or soon after being placed in the oviposition vials. Also, many died on the water, apparently attempting to oviposit. As Table 2 indicates, the control mosquitoes died at a relatively constant rate, and no unusual pattern of mortality was observed.

LT-50's and LT-90's computed from the daily mortality data are presented in Table 3. The 51% reduction in longevity of females exposed as 1st instar larvae was ca. the same as that reported earlier (Anthony et al. 1972). The reduction of 39% for the females exposed as 3rd and 4th instar larvae and the calculated reductions of 45 and 46% in F_1 progeny (based on the percentage of eggs hatched compared with the percentage of eggs hatched in the control) show that *N. algerae* had a marked effect on the longevity and fecundity of *An. albimanus*, even when infections were induced in the late instars.

INFECTIONS IN EGGS. Varying numbers of eggs that failed to hatch were found to

Table 3. Effects of *Nosema algerae* on the longevity of *Anopheles albimanus* when infections were induced in different larval instars.

Instars Infected	LT-50 (days)	% Reduction in Longevity Compared to controls	LT-90 (days)	% Reduction in Longevity Compared to controls
1st	12.2	45	18.9	51
2nd	11.7	47	16.5	57
3rd	14.8	33	23.6	39
4th	15.1	33	23.7	39
Uninfected controls	22.2		38.7	

be infected with *N. algerae*. Infected eggs were found at the 1st gonotrophic cycle in all groups except those exposed as 4th instar larvae, and infections were found in the eggs of this group too at the 2nd and succeeding cycles. Infection rates of the unhatched eggs ranged from 10 to 70% and appeared to be related to the intensity of the *Nosema* infection in the parent female.

We were unable to determine whether the number of infected eggs increased at each gonotrophic cycle because the females selected for study invariably died before completing the next cycle. However, the number of eggs laid and the percentage hatch definitely declined in each cycle after the 1st, and these reductions were noted in all groups. The number of eggs laid by control females also declined in the later gonotrophic cycles, but the reductions were not so great, and the percentage of hatch was always 80% or higher.

Nearly all of the infected females laid infected eggs; however, we could not demonstrate transovarial transmission of *N. algerae* to the F_1 progeny when the eggs were washed by a method similar to that described by Ford and Green (1972). Also, many newly hatched larvae were examined by making Giemsa-stained smears, and evidence of infection by *N. algerae* was never found. These findings are in agreement with those of Canning and Hulls (1970) who concluded that, on the whole, infected eggs did not develop into viable larvae. However, the eventual decay of infected eggs and the external contamination by spores on viable eggs laid by infected females may be natural sources of infection to newly hatched larvae.

Nosema infections have also been shown to reduce the longevity and fecundity of certain Lepidoptera (Zimmack et al. 1954, Zimmack and Brindley 1957 and Veber and Jasic 1961). Studies of longevity and the reproductive and diapause potential of *Heliothis zea* (Boddie) infected with *Nosema heliothidis* Lutz and Splendore were reported by Gaugler and Brooks (1974). When compared with uninfected individuals, the infected specimens showed significant reductions in longevity, fecundity and mating success. Also in the laboratory, fewer of the infected pupae initiated diapause and the intensity of diapause was decreased.

Our tests indicate that the effects of *N. algerae* on *An. albimanus* may be similar to those reported for *Nosema* species and their lepidopteran hosts. Undeen and Alger (1974) found that infections of their isolate of *N. algerae* significantly reduced the longevity of *An. stephensi* Liston; however, *An. albimanus* was not so severely affected. These workers indicated that the difference may have resulted from selection since their isolate had been maintained in *An. stephensi* for 7 years. Similarly, the isolate (El Salvador) of *N. algerae* used in our studies had been maintained primarily in *An. albimanus* (with periodic passages through *H. zea*) since 1971. However, many bioassay tests at our laboratory have shown that there are only slight differences in the susceptibility of *An. albimanus* and *An. quadrimaculatus* Say to the El Salvador isolate of *N. algerae*.

Our studies suggest that we should look beyond immediate larval or adult mortality in assessing the effects of chronic diseases. The highest larval mortality observed in the tests was 14% (group exposed as 1st instar larvae, 1st replicate), but the effects of the disease on the ensuing adults were highly significant at all parameters observed. The calculated reductions in the number of eggs hatched (Table 1) indicate that the introduction of *N. algerae* into populations of *An. albimanus* consisting of mixed larval instars could result in an average reduction of F_1 progeny of about 54% from those females that become infected. We believe that the successful introduction of *N. algerae* into natural breeding areas of *An. albimanus* could reduce densities of adults and thus would substantially reduce the rate of malaria transmission.

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