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The effect of nosematosis on the development of *Plasmodium falciparum* in *Anopheles stephensi**

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Abstract. To quantify the effect of *Nosema algerae* (Microsporida, Nosematidae) on the development of *Plasmodium falciparum* in *Anopheles stephensi* (Diptera, Culicidae), we carried out infection experiments under standardized laboratory conditions. Apart from a mean reduction of 69% in oocyst development, smaller numbers of oocysts and fewer sporozoites were found in the *Nosema*-infected mosquitoes. In addition, nosematosis resulted in higher mortality. The potential role of *Nosema algerae* as a biological control agent is discussed.

Since 1958, the development of malaria parasites in mosquitoes infected with Microsporidia has been investigated by several authors using different mosquito species and various *Plasmodium* strains (Bano 1958; Fox and Weiser 1959; Hulls 1971; Savage et al. 1972; Ward and Savage 1972; Gajanana et al. 1979; Schenker et al. 1991). The effect of nosematosis in the various investigations ranged from "no significant differences... in mean malarial oocyst counts" between *Nosema*-infected and control groups of *Anopheles quadrimaculatus* that were infected with *P. cynomolgi* (Ward and Savage 1972) to inhibition of *P. falciparum* development in *A. gambiae* that had been heavily infected with *Nosema* (Fox and Weiser 1959). In general, however, a reduction in the development of malaria parasites was noticed.

Schenker and colleagues (1990) first carried out "double" infection experiments under standardized laboratory conditions using *A. stephensi*, *P. yoelii nigeriensis*, and *N. algerae*. The purpose of the present study was to quantify the effect of nosematosis (*N. algerae*) on the development of *P. falciparum* in *A. stephensi* under standardized laboratory conditions.

The *Nosema* strain used in the present experiments was detected in our mosquito colony in 1986 by Bamberger and was identified as *N. algerae* by morphological criteria. The *A. stephensi* colony used in this study was pooled from three different strains from London, Basel, and Zürich (obtained from Dr. B. Merkli, Basel). The rearing conditions and the mode of infection with *Nosema* have previously been described (Schenker et al. 1991). For the present experiments, the method was slightly modified in that 800 first-instar larvae were infected by the application of 2×10^6 Microsporidia, i.e., 5,600 spores/cm^2 (2×10^4 spores/ml). All larvae were fed with a standardized quantity of powdered fish food (Tetra tabimin/Tetrawerke) until pupation occurred.

Gametocytes of the *P. falciparum* strain NF54 (kindly supplied by Dr. R. Ponnudurai, Nijmegen) were produced according to modified methods of Ponnudurai and colleagues (1982), Schneweis and co-workers (1991), and Brockelman (1982). The hematocrit in the cultures was adjusted to 7%. On day 3 of cultivation, the culture medium was supplemented with 20% erythrocyte lysate (Schneweis et al. 1991). After 4 days, the cultures were divided into two dishes (Brockelman 1982).

Nosema-infected and *Nosema*-free *A. stephensi* aged 3–5 days were infected by membrane feeding (Ponnudurai et al. 1989) with gametocytes that had been harvested at 14 or 15 days after the initiation of the cultures. Unfed mosquitoes were removed. In some experiments, the unfed *Nosema*-infected mosquitoes were homogenized and the mean number of spores per mosquito were determined using a Neubauer counter. A second blood meal was given on day 5 following the infective one. Dead mosquitoes were counted daily in both groups. At 8–10 days postinfection (p.i.), the midguts were examined for oocysts and microsporidian infections. In one experiment it was possible to check the salivary glands for sporozoites.

The rate of *Nosema* infection in the different experiments varied between 31% and 100% (Table 1). The low level of 31% may have been caused by the high mortality that was observed until day 10 after membrane feeding (69%; individual data not shown). In accordance with previous studies, the concomitantly infected

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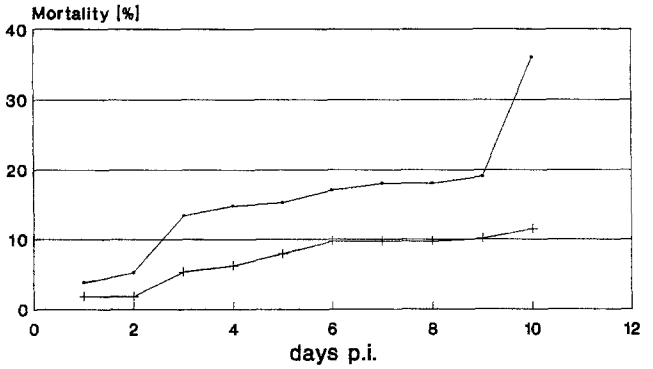

Fig. 1. Mortality of *Nosema*-infected (–■–) and *Nosema*-free (–+–) *Anopheles stephensi* following subsequent infection with *Plasmodium falciparum*

females used in these experiments also showed higher mortality than the control group. In Fig. 1, the mean mortality noted for eight infection experiments is summarized. By day 10 after membrane feeding, 36% of the *Nosema*-infected females had died as compared with 11.5% of the control group. These results agree with those previously reported by Schenker and colleagues (1991), who observed mortality amounting to 32% in the *Nosema*-infected group vs 22.7% in a noninfected group. The much higher mortality of 61.4% that was noted by these authors in double-infected mosquitoes might have been attributable to the finding that an infection with *P. y. nigeriensis* causes higher mortality in mosquitoes (41.2%) than does a *P. falciparum* infection.

The gut-infection rates recorded on day 10 after membrane feeding are listed in Table 1. Overall, only 12 of 124 (9.7%) of the *Nosema*-infected group developed oocysts, whereas 55 of 176 (31.3%) of the control group showed oocyst development. This indicates that on average, oocyst development in *Nosema*-treated anophelines was reduced by 69% as compared with untreated controls. The mean number of oocysts per positive gut were lower in *Nosema*-infected females than in the control group (5 and 16, respectively; Table 2). As the data in Table 3 demonstrate, the frequency distribution of oocysts differed between the test groups; only one

Table 2. Number of *Nosema*-infected females, mean spore number per mosquito, and average number of oocysts in *Nosema*-infected and control mosquitoes

Culture number	*Anopheles stephensi*			
	Nosema-infected			Controls
	% Females *Nosema*-positive	Mean spore number mosquito	Oocysts/ positive gut ($n \pm$SE)	Oocysts/ positive gut ($n \pm$SE)
NF13	63	ND	0	3± 0.3
NF3	93	ND	0	11± 2.4
NF7	80	ND	0	1
NF4	86	ND	6.5±2.3	35±10
NF2	31	ND	0	1
NF9	100	1.1×10^6	1	1.5
NF11	100	2.1×10^6	4±0	12± 2.4
NF12	100	2.1×10^6	3±1	22± 5.1
Totals			5±0.9	16± 4.3

ND, Not done

Table 1. Gut infection and salivary-gland infection of *Nosema*-infected and *Nosema*-free control *Anopheles stephensi* fed on gametocytes of *Plasmodium falciparum*

Culture number	Gametocytes day 13/14 (stage V) %	*Anopheles stephensi*					
		Nosema-infected				Controls	
		Positive/ examines (n)	%	*Nosema*-positive %	Mean spore number/ mosquito	Positive/ examined (n)	%
NF13	2.8	0/19	0	63	ND	5/29	7
NF3	1.7	0/14	0	93	ND	9/19	47
NF7	4.0	0/10	0	80	ND	1/27	3.7
NF4	2.2	6/21	28.6	86	ND	12/22	54.5
NF2	1.2	0/16	0	31	ND	2/30	6.7
NF9	2.6	1/26	3.8	100	1.1×10^6	3/25	12
NF11[a]	5.9	1/11	9	100	2.1×10^6	15/16	94
NF12[a]	4.3	4/7	57	100	2.1×10^6	8/8	100
Total**		12/124	9.7			55/176	31.3
Salivary-gland infection:							
NF11[a]	5.9	1/4	25	100	2.1×10^6	16/16	100
NF12[a]	4.3	–	–	100	2.1×10^6	8/9	89

** According to the sign test (Dixon and Monod), these differences are highly significant ($P = 0.004$)
ND, Not done
[a] Tested mosquitoes belonging to the same batch

Table 3. Frequency distribution of oocysts of *Plasmodium falciparum* in *Nosema*-infected and control mosquitoes

Oocysts/ mosquito	*Anopheles stephensi*			
	Nosema-infected		Controls	
	Total	%	Total	%
0	112	90.3	121	68.8
0–10	11	8.9	26	14.8
11–20	1	0.8	15	8.5
21–30	–	–	8	4.5
31–50	–	–	3	1.7
50–100	–	–	2	1.1
>100	–	–	1	0.6
Total	124		176	

of the *Nosema*-infected mosquitoes exhibited more than ten oocysts. These results confirm the data of Schenker et al. (1991). In their experiments, the oocyst development of *P. y. nigeriensis* was reduced by 33.2% in *Nosema*-treated anophelines. Furthermore, they found a mean reduction of 84.68% in the number of oocysts in *Nosema*-infected *A. stephensi*.

As described elsewhere (Ward and Savage 1972; Schenker et al. 1991), it is difficult to obtain a sufficient number of mosquitoes for sporozoite examination because of the high mortality of dual-infected mosquitoes. In the present study, checking the salivary glands of both groups for sporozoites was possible in only one experiment. Only one of four dissected *Nosema*-infected females displayed a few sporozoites in the salivary glands, whereas in the control group, sporozoites were found in all mosquitoes dissected (100%; see Table 1). In the latter group, the sporozoite content per mosquito as visualized by microscope was much higher than that in the *Nosema*-infected group. The exact numbers of sporozoites were not recorded.

Bano (1958) concluded that the partial inhibitory effect of *Plistophora culicis* on the sporogonic cycle of *P. cynomolgi* may be due to the scarcity of essential nutriments or to an abnormality in the function of heavily infected Malpighian tubules followed by the deposition of toxic substances. One experiment was carried out to ascertain the influence of the lack of nutrition. Females in both test groups were fed with normal blood at 72 h prior to the infective blood meal. Unfed mosquitoes were removed. The mean number of spores in the *Nosema*-infected group were 2×10^6/mosquito. In all, 6 of 12 (50%) prefed *Nosema*-infected females developed oocysts, for an average of 2 oocysts/positive gut. In the control group, 9 of 12 (75%) females examined for gut infection were found to be positive for *Plasmodium*, for an average oocyst count of 22. The reduction in *Plasmodium* development observed in this experiment was lower than the previously reported values yet amounted to 33%. Moreover, the number of oocysts were decreased. Therefore, it can be assumed that lack of nutrition is only partly responsible for the inhibition of *Plasmodium* development in *Nosema*-infected mosquitoes.

In all, 11 of 16 (69%) mosquitoes in the control group that were examined for sporozoites proved to be positive. In the microsporidian-infected group, only one mosquito survived for salivary-gland dissection, which revealed very few sporozoites. Evidently, sporozoites from *Nosema*-infected mosquitoes are less infective (Hulls 1971; Maier, unpublished data). Thus, it can be expected that transmission of malaria might be hampered not only by a reduction in sporozoite numbers but also by a decrease in the viability of the sporozoites. Furthermore, Rickman and colleagues (1990) found that not every bite of a *P. falciparum*-infected mosquito transmitted malaria; exposure to one or two infected mosquitoes led to parasitemia in only 50% of the volunteers.

Fox and Weiser (1959) attributed the inhibition of *P. falciparum* development to the disintegration of the midgut wall in *A. gambiae* that had been heavily infected with *Nosema*. Unfortunately, these authors did not precisely define "heavily infected." However, in our experiments, the midgut wall of mosquitoes that had been infected with 2×10^{-6} spores had not disintegrated enough to prevent oocysts development completely.

Some authors (Anthony et al. 1972; Gajanana et al. 1979; Geetha Bai et al. 1979; Savage et al. 1972) consider *N. algerae* to be a promising biological control agent due to its capability of diminishing the vector capacity of malaria-transmitting mosquitoes by increasing the mortality of the mosquitoes during all stages of their life cycle, by decreasing the reproductivity and longevity of the adult females, and by disturbing the development of the malaria parasites within the vector. Undeen and Alger (1975) doubt the effectiveness of *N. algerae* as a larvicide but attribute the lower rates of malaria transmission to a drastic reduction in the life span of adult mosquitoes. In spite of the promising results obtained under laboratory conditions, Haq et al. (1981) judge the use of *Nosema* under field conditions as being worthless. This finding contrasts with a field test carried out by Anthony et al. (1978), which resulted in infection rates of between 16% and 86% in *A. albimanus*, depending on the infection dose.

On the basis of laboratory tests, Anthony et al. (1972) constructed a population model to estimate theoretically the effect of *N. algerae* on adult *A. albimanus*. Using this model, they calculated that a microsporidian infection dose of 5×10^4 spores/ml, which reduced the longevity of females by one-half, would diminish the percentage of infective females by 85%–97%. Considering that the development of malaria parasites is inhibited by an average of about 69%, the number of infective females would be even more drastically reduced. In combination with an effective application method, *N. algerae* might be useful as an additional biological agent in integrated malaria control.

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References

Anthony DW, Savage KE, Weidhaas DE (1972) Nosematosis: its effect on *Anopheles albimanus* Wiedemann and a population model of its relation to malaria transmission. Proc Helminthol Soc Wash 39 [Suppl]:428–433

Anthony DW, Savage KE, Hazard EI, Avery SW, Boston MD, Oldacre SW (1978) Field tests with *Nosema algerae* Vavra and Undeen (Microsporida, Nosematidae) against *Anopheles albimanus* Wiedemann in Panama. Miscellaneous Publications of the Entomological Society of America 11. p 17–27

Bamberger K (1986) Experimentelle Untersuchungen zum Entwicklungszyklus von *Vavraia culicis* (Microsporida) in *Anopheles*. Diplomarbeit, Universität Bonn

Bano L (1958) Partial inhibitory effect of *Plistophora culicis* on the sporogonic cycle of *Plasmodium cynomolgi* in *Anopheles stephensi*. Nature 181:430

Brockelman C (1982) Conditions favoring gametocytogenesis in the continuous culture of *Plasmodium falciparum*. J Protozool 29:454–458

Fox RM, Weiser J (1959) A microsporidian parasite of *Anopheles gambiae* in Liberia. J Parasitol 45:21–30

Gajanana A, Tewari SC, Reuben R, Rajagopalan PK (1979) Partial suppression of malaria parasites in *Aedes aegypti* and *Anopheles stephensi* doubly infected with *Nosema algerae* and *Plasmodium*. Indian J Med Res 70:417–423

Geetha Bai M, Das PK, Gajanana A, Rajagopalan PK (1979) Host-parasite relationship of *Nosema algerae*, a parasite of mosquitoes. Indian J Med Res 70:620–624

Haq N, Reisen WK, Aslamkhan M (1981) The effects of *Nosema algerae* on the horizontal life table attributes of *Anopheles stephensi* under laboratory conditions. J Invertebr Pathol 37:236–242

Hulls RH (1971) The adverse effects of a microsporidian on the sporogony and infectivity of *Plasmodium berghei*. Trans R Soc Trop Med Hyg 65:421–422

Ponnudurai R, Meuwissen JHET, Leeuwenberg ADEM, Verhave JP, Lensen AHW (1982) The production of mature gametocytes of *Plasmodium falciparum* in continuous cultures of different isolates infective to mosquitoes. Trans R Soc Trop Med Hyg 76:242–250

Ponnudurai R, Lensen AHW, Gemert GJA van, Bensink MPE, Bolmer M, Meuwissen JHET (1989) Infectivity of cultured *P. falciparum* gametocytes to mosquitoes. Parasitology 98:165–173

Rickman LS, Jones TR, Long GW, Paparello S, Schneider I, Paul CF, Beaudoin RL, Hoffmann SL (1990) *Plasmodium falciparum*-infected *Anopheles stephensi* inconsistently transmit malaria to humans. Am J Trop Med Hyg 43:441–445

Savage KE, Lowe RE, Hazard EI, Lofgren CS (1972) Studies on the transmission of *Plasmodium gallinaceum* by *Anopheles quadrimaculatus* infected with a *Nosema* sp. WHO/VBC/70.237. WHO, Geneva

Schenker W, Margos G, Chutmongkonkul M, Maier WA, Seitz HM (1990) The effects of *Nosema algerae* on the development of *Plasmodium* in *Anopheles* mosquitoes. Zentralbl Bakteriol Mikrobiol Hyg [A] 317:63

Schenker W, Maier WA, Seitz HM (1991) The effects of *Nosema algerae* on the development of *Plasmodium yoelii nigeriensis* in *Anopheles stephensi*. Parasitol Res (in press)

Schneweis S, Maier WA, Seitz HM (1991) Hemolysis of infected erythrocytes – a trigger for gametocyte formation of *Plasmodium falciparum*? Parasitol Res 77:458–460

Undeen AH, Alger NE (1975) The effect of the microsporidian, *Nosema algerae*, on *Anopheles stephensi*. J Invertbr Pathol 25:19–24

Ward RA, Savage KE (1972) Effects of microsporidian parasites upon anopheline mosquitoes and malarial infection. Proc Helminthol Soc Wash 39 [Suppl]:434–438