LACK OF SUSCEPTIBILITY OF MICE AND RATS TO THE MOSQUITO NEMATODE *REESIMERMIS NIELSENI* TSAI AND GRUNDMANN

C. M. IGNOFFO,[1] J. J. PETERSEN,[2] H. C. CHAPMAN [2] AND J. F. NOVOTNY [3]

ABSTRACT. Suckling and adult mice and adult rats were subjected to either *per os, intranasal, intraperitoneal,* or *dermal* challenge of the mosquito nematode *Reesimermis nielseni.* Body weight gain and histological examination of tissue from animals receiving nematodes were essentially identical to those of untreated animals. Immunodepressed rats also were not susceptible. A few dead nematodes were detected in feces from mice fed nematodes. Nematodes were not detected in urine samples.

The nematode *Reesimermis nielseni* Tsai and Grundmann is a potential biological control agent of culicine and anopheline mosquitoes (Petersen, 1973). The normal application technique is to introduce living preparasitic larvae into aquatic habitats suspected of harboring populations of mosquitoes. The impact of this introduction, both on man and the environment, should be carefully considered and evaluated (Ignoffo, 1973).

The literature from 1880 to 1963 cites five observations of insect mermithids (*Agamomermis* spp.) associated with humans (Leidy, 1880; Stiles, 1908; Stiles and Hassal, 1926; Baylis, 1927; Neveu-Lemaire, 1936; Leon, 1946; Watson, 1960; Foster, 1963). In each citation, the mermithid was associated with the alimentary or urinary tract. Foster (1963) concluded that *Agamomermis* probably occurs in man only as an accidental parasite, probably enters via the mouth and . . . "that the infection is relatively benign if confined to the gut," . . . however . . . "clinical symptoms are produced if other organs are involved." Direct experimentation to induce infestation and development of insect mermithid nematodes in animals is one way of refuting or corroborating reports of "accidental" parasitism.

Ignoffo *et al.* (1973) demonstrated that preparasitic larvae of *R. nielseni* are specific for mosquitoes. Neither rainbow trout, channel catfish, large mouth bass, fat head minnow, top minnow, nor any of 16 invertebrate species supported development of *R. nielseni.* Our present study evaluated the susceptibility of mammals, i.e., white rats and mice, to an administration of preparasitic larvae of *R. nielseni.* This, to our knowledge, is the first time an evaluation of the susceptibility of mammals to an endoparasitic, biological-control agent has been published.

METHODS AND MATERIALS

EXPERIMENTAL ANIMALS. Infective-stage larvae of *R. nielseni* hatched and were collected from cultures of eggs laid in moist sand (Petersen and Willis, 1972; Petersen, 1973). The sand and eggs were flooded with water for 2–4 hrs. Hatched flooded were decanted, washed in distilled water, concentrated on filter paper, resuspended in a known volume of water, and then counted. Counts, made under a stereoscopic microscope were recorded as number of preparasitic larvae/ml. Counts of concentrated larvae averaged 2000–3000 nemas/ml.

The Fort Detrich strain of white albino mice and the Charles River strain of white rats were used as experimental hosts. Adult mice weighed 46.8 g/mouse; suckling mice 1, 2, or 3 days old averaged 2.4, 2.6, and 2.9 g/mouse, respectively; and white rats averaged 248.5 g/rat. All ex-

[1] Biological Control of Insects Research, ARS, USDA, North Central Region, Columbia, Missouri 65201.
[2] Gulf Coast Mosquito Research Laboratory, ARS, USDA, Southern Region, Lake Charles, Louisiana 70601.
[3] Woodard Research Corporation, Herndon, Virginia 22070.

perimenal animals were provided food and water *ad libitum*.

EXPERIMENTAL PROCEDURE. Freshly collected aqueous suspensions of preparasitic larvae were administered to adult mice by intranasal insertion, intraperitoneal injection, and *per os* (by mouth). Five mice were used for each administration route. Only distilled water was given to the control mice. The average number of larvae and the volume/mouse for each administration route was: intraperitoneal, 1063.8 nematodes in 0.47 ml of water; intranasal, 782.0 nematodes in 0.34 ml; *per os,* 1057.6 nematodes in 0.46 ml. Three-day-old, suckling mice also received an intraperitoneal injection, intranasal insertion, and *per os* administration of 70 nematodes per 0.03 ml of water. In a second test series, 10, 15, and 5 suckling mice 1, 2, and 3 days old, respectively, received intraperitoneal injections of 125 nematodes/mouse. Suckling mice were included, because they have a low level of natural immunity and could be more susceptible to nematode infestations. Urine and fecal samples from both adult and suckling mice were collected on days 1, 2, 3, 7, 14, and 21 post-challenge and were examined microscopically for the presence of nematodes. Weekly weights were also recorded for each mouse. All mice were sacrificed and examined for gross pathological effects after 21 days. Tissues in which abnormalities were observed or tissues obtained from animals which died during the test were subjected to histopathological examination.

The ability of nematodes to infest white rats following dermal administration was also investigated. The back and flank of six adult rats were shaved and slightly scratched to produce an abrasion. Three rats were injected (5 mg/kg) with corticosteroid prednisolone (CSP), and all six rats then received 460 nematodes/rat in a volume of 0.2 ml of water. Applications of *R. nielseni* were made at 1, 3, and 5 days after injection with CSP. Corticosteroid prednisolone interferes with the normal immunological resistance of rats exposed to species of Aschelminthes (Novotny, unpublished). We included the steroid treatment in an attempt to put the rats in an immuno-depressed state and hopefully make them *more* susceptible to *R. nielseni*. Blood samples were drawn before treatment with nematodes to establish base levels of white blood cell and differential cell counts and again 7, 14, and 21 days after treatment. Urine and fecal samples were collected at 0, 7, 14, and 21 days post-challenge and microscopically examined for nematodes. After 21 days, the rats were sacrificed and tissues, i.e. liver, lung, heart, small intestine, colon, kidney, spleen, lymph nodes, and salivary gland, were examined for evidence of infestations or damage due to nematodes.

RESULTS AND DISCUSSION

Two of the adult mice and two of the suckling mice receiving intraperitoneal injections of nematodes died the 1st and 2nd day after injection, respectively. Histological examination of tissue from these mice, as well as from mice sacrificed at the end of the test (21 days), disclosed no nematodes or changes attributable to nematodes. Body weight gains of nematode-treated adult mice were essentially similar to those of the injected controls (Table 1). Suckling mice (1st series, intraperitoneal injections of 70 nematodes/mouse), however, did not gain weight as uniformly as those receiving the other treatments. Death or failure to show normal weight gains were not observed when a second series of 1, 2, or 3 day old suckling mice were intraperitoneally injected with 125 nematodes/mouse (Table 1). Histological examination of all intraperitoneally-injected mice after the 1st or 2nd series of tests was concluded showed no evidence of nematode presence or damage of mouse tissue. Nematodes were not detected in urine samples taken from adult or suckling mice. A few dead nematodes were observed (1 and 2 days post-treatment) in feces taken from adult mice receiving *per os* administered nematodes.

TABLE 1. Weight gains in adult and suckling mice administered infective-stage larvae of the mosquito nematode *Reesimermis nielseni*.[a]

Administration route	Avg. nematodes per mouse	Avg. nematodes per gram/mouse	Avg. body weight (g) Start	Avg. body weight (g) End
Adult mice				
Intraperitoneal	1063.8	22.8	40.8	46.3
Intranasal	782.0	23.0	32.0	36.0
Per os	1057.6	23.0	46.0	49.8
Intraperitoneal Ck[b]	0.0	0.0	31.4	33.0
Suckling mice				
Intraperitoneal[c]	70.0	25.0	2.8	10.0
Intraperitoneal[d]				
1-day-old	125.0	50.0	2.5	21.9
2-day-old	125.0	48.1	2.6	22.3
3-day-old	125.0	41.6	2.9	22.0
Intranasal	70.0	25.0	2.6	19.6
Per os	70.0	25.0	3.0	21.6
Intraperitoneal Ck[b]	0.0	0.0	2.6	23.4

[a] Five animals were used for each route of administration except for the 1 or 2 day old suckling mice where 10 and 15 mice were used, respectively.
[b] Treated checks injected with distilled water.
[c] First series: only 3-day-old mice were used.
[d] Second series: 1-, 2-, or 3-day-old mice were used.

Nematodes were not detected in fecal samples from adult or suckling mice treated intranasally or intraperitoneally.

Results of studies of dermal treatment of rats with nematodes also were negative. No evidence of nematode penetration into the skin or the systemic presence and survival of nematodes was detected. Urine and fecal samples taken immediately after challenge and again at 7, 14, and 21 days after treatment also were negative. Histological examination of rat tissue at the conclusion of the test did not reveal nematodes or damage due to nematodes. The levels of white blood cells, granulocytic cells, lymphocytes, and monocytes were all within the normal range for white rats (Schermer, 1967). In addition, no differences in responses to nematodes could be detected between corticosteroid prednisolone-treated rats and the non-steroid-treated rats.

The results of our studies, as well as those previously documented from other vertebrates and invertebrates (Petersen, *et al.*, 1968; Ignoffo, *et al.*, 1973), indicate that the parasitic nematode *R. nielseni* is apparently host specific for mosquitoes.

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OCCURRENCE OF TWO TYPES OF GYNANDROMORPHISM IN A SIBLING SERIES OF *AEDES* (*STEGOMYIA*) *CRAGGI* (BARRAUD) (DIPTERA: CULICIDAE)[1]

YIAU-MIN HUANG

Medical Entomology Project, Department of Entomology, Smithsonian Institution, Washington, D.C. 20560

ABSTRACT. Anterior-posterior and bilateral gynanders of *Aedes* (*Stegomyia*) *craggi* (Barraud) were encountered in a sibling series of this species. The occurrence of two types of gynandromorphism within such a series of mosquitoes has not been previously reported. Reports of mosquito gynandromorphs subsequent to, or not included in the tabulation of Brust (1966) are summarized.

Two gynandromorphs of *Aedes* (*Stegomyia*) *craggi* (Barraud) were found among the *Stegomyia* mosquitoes submitted by the SEATO Medical Research Laboratory, Bangkok. Both specimens were reared from eggs obtained from a wild-caught female biting man in a forest at Chiang Mai, Thailand. It is noteworthy that these 2 gynandromorphs are of 2 distinct types known as an anterior-posterior gynander and a bilateral gynander. Accompanying these 2 gynanders are another 12 specimens (7 males, 5 females) which were derived from the same female (mother). These are normal siblings. As far as it can be determined, this is the first time that 2 types of gynandromorphism have been reported from the same sibling series. Craig and Hickey (1969:102) report 4 gynanders in one sibship of 16 individuals but do not indicate if 2 or more types were represented. The two gynanders are as follows:

(1). Anterior-posterior gynander, specimen No. (1)–2 with associated terminalia on slide (SEAMP 345, 73/302). This specimen has antennae, male; palpi, male; fore- and midtarsal claws unequal, male; hindtarsomere 4 with basal 5/6 white and tarsomeres 3, 5 dark, female; abdomen and genitalia, female, normal, all three spermathecae present.

(2). Bilateral gynander, specimen No. (1)–5 with associated terminalia on slide (SEAMP 345, 73/301). This specimen has right antenna, male; right palpus, male; right fore- and midtarsal claws unequal, male; right hindtarsomeres 3–5 dark, male; left antenna, female; left palpus, female; left fore- and midtarsal claws equal, female; left hindtarsomere 4 with

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