

Zeitschrift:	Acta Tropica
Herausgeber:	Schweizerisches Tropeninstitut (Basel)
Band:	42 (1985)
Heft:	4
Artikel:	Site of resistance to "Necator americanus" in hamsters
Autor:	Rajasekariah, G.R. / Deb, B.N. / Dhage, K.R.
DOI:	https://doi.org/10.5169/seals-313484

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften auf E-Periodica. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. Das Veröffentlichen von Bildern in Print- und Online-Publikationen sowie auf Social Media-Kanälen oder Webseiten ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. [Mehr erfahren](#)

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. La reproduction d'images dans des publications imprimées ou en ligne ainsi que sur des canaux de médias sociaux ou des sites web n'est autorisée qu'avec l'accord préalable des détenteurs des droits. [En savoir plus](#)

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. Publishing images in print and online publications, as well as on social media channels or websites, is only permitted with the prior consent of the rights holders. [Find out more](#)

Download PDF: 29.01.2026

ETH-Bibliothek Zürich, E-Periodica, <https://www.e-periodica.ch>

Hindustan Ciba-Geigy Research Centre, Goregaon East, Bombay 400063, India

Site of resistance to *Necator americanus* in hamsters*

G. R. RAJASEKARIAH, B. N. DEB, K. R. DHAGE, S. BOSE

Summary

Resistance to the development of human hookworm, *Necator americanus* was examined in 3- to 6-week-old young adult hamsters. Only 3% of *N. americanus* infective third stage larvae (NaL₃) reached maturity in the intestines of young adults as opposed to as many as 60% in 2-day-old baby hamsters. This seemingly effective resistance prevailing in young adults was investigated in some detail. The skin, the first site of contact for the invading NaL₃, was bypassed during the infection process. Completely in vitro exsheathed NaL₃ (ExNaL₃) were used, and young adult hamsters were infected parenterally, bypassing the skin. Even after exsheathing the larvae artificially before infection and bypassing the skin, no improvement was seen in the development of *N. americanus* in the intestines of young adults. Higher infection doses also did not increase the worm burden. Some of the factors limiting the development of parasites in young adults were examined. *N. americanus* were monitored in lungs and intestines during various intervals after infection. Similar parasite burdens were apparent in lungs of baby as well as young adult hamsters. In the intestines, a significantly lower burden of *N. americanus* was seen during various intervals in young adults compared to the baby hamsters. Moreover, *N. americanus* were expelled soon after reaching the intestine. This comparative monitoring revealed the intestine as the seat of resistance against the establishment of *N. americanus* in young adult hamsters.

Key words: *Necator americanus*: in vitro exsheathment; baby hamsters; young adult hamsters; age resistance; immunology; tissue migration; worm recovery; lungs; intestine

* Communication No. 739 of Hindustan Ciba-Geigy Research Centre

Correspondence: Dr. G. R. Rajasekariah, Head, Biology of Tropical Diseases, Hindustan Ciba-Geigy Research Centre, Goregaon (E), Bombay 400063, India

Introduction

There is no suitable animal model with which to study the immunology of the human hookworm, *Necator americanus*. Sen and Seth (1967) used 2-day-old baby hamsters which are susceptible and in which *N. americanus* undergoes complete development. However, not many immunological studies are possible in baby hamsters because they have to be infected as early as 2 days after their birth. Moreover, as many as 40% of infected babies die due to several reasons (Rajasekariah et al., 1985). Three- to 6-week-old young adult hamsters are therefore preferred but they are generally regarded as "resistant" (Ogilvie et al., 1975). However, no comparative data are available on the extent of establishment of *N. americanus* in hamsters of different age groups. In view of the limitations in using baby hamsters, there is a need to study the natural resistance and to pinpoint the possible site(s) of resistance against the establishment of *N. americanus* in young adult hamsters. Some experiments conducted on these lines are reported here.

Materials and Methods

Hamsters. Two-day- (baby) and 3- and 6-week-old (young adult) golden hamsters (*Mesocricetus auratus*) were used for infection. After infection, baby hamsters were maintained with their mothers until weaning on day 21. The maintenance and handling of hamsters during the period of study are described elsewhere (Rajasekariah et al., 1985).

*Harvesting ensheathed infective larvae of *N. americanus* (EnNaL₃): their exsheathment in vitro and infection of hamsters.* The production of ensheathed infective third stage larvae of *N. americanus* (EnNaL₃) is described elsewhere (Rajasekariah et al., 1985). The exsheathment of EnNaL₃ was performed in vitro. EnNaL₃ were washed 3 times in tissue culture medium (RPMI 1640, GIBCO, with Hepes and bicarbonate buffer pH 7.2, antibiotics and supplemented with 1% glucose) and then transferred into a glass tube (diameter 1.5 cm, length 10 cm) one end of which was closed by tying the freshly dissected skin of 3-week hamsters on to its outer rim. Care was taken to avoid any leakage. The tube containing known numbers of EnNaL₃ in 2 ml medium was suspended with a clamp into an external container (25 ml glass beaker) with 10 ml of medium with the closed end of the tube covered with skin fully immersed in the medium. The whole system was incubated at 37° C for 24 h under sterile conditions. During the incubation period EnNaL₃ penetrated the skin, completely exsheathed and were liberated into the external container. The exsheathment was checked under the microscope and the larvae in the external container designated "exsheathed NaL₃" (ExNaL₃). EnNaL₃ and ExNaL₃ were washed briefly 3 times in warm (37° C) tissue culture medium. Active larvae were counted on a heat-stage Leitz microscope and used for infection. Baby hamsters were infected percutaneously under anaesthesia (Sen and Seth, 1967). Young adult hamsters were infected 1. percutaneously by exposure to EnNaL₃ suspended in distilled water in a wide-mouth screw-cap bottle and 2. by administering EnNaL₃ intraperitoneally, intrathoracically and per os.

*Assessment of development of *N. americanus* in hamsters.* The number, type and the dose of NaL₃ used for infection and the time of sacrificing infected hamsters are mentioned in the Results section. NaL₃ were recovered from the lungs 4 to 6 days after infection. The lungs from each animal were chopped into 2 mm cubes and transferred on to a sieve (mesh size 1 mm²) which fitted into a glass dish containing 25 ml sterile tissue culture medium. They were incubated at 37° C for 3–4 h (during incubation, the larvae migrate from the tissue and settle to the bottom of the dish). After

Table 1. Development of *Necator americanus* in naïve baby and young adult hamsters

Hamster	Generation number of NaL ₃ *	No. of <i>N. americanus</i> collected from the small intestine of hamsters 37 days after infection each with 100 EnNaL ₃ , percutaneously	Development
Age	No. survived/ No. used	Individual recovery	Mean ± SD
Baby	15/25	HS-68	18, 24, 28, 34, 35, 38, 41, 45, 47, 50, 60, 65, 66, 69, 79
Young adult	10/10	HS-68	0, 0, 1, 1, 1, 2, 3, 5, 9

* NaL₃ used were derived from the 68th generation of human hookworm, *N. americanus*, adapted to hamster
Statistical analysis: b < a; P < 0.001 (Mann-Whitney U-test)

Table 2. Numbers of EnNaL₃ migrated through the skin of young adult hamsters during different intervals in vitro

Skin No.	No. of EnNaL ₃ transferred on to the upper surface of the hamster skin	No. of ExNaL ₃ collected at different intervals (in hours) during incubation in vitro				Total No. of ExNaL ₃ recovered from each skin
		1	4	6	8	
<i>Experiment 1</i>						
Skin 1	2000	0	250	50	250	50
Skin 2	2000	0	100	50	50	250
Skin 3	2000	0	200	50	50	600
<i>Experiment 2</i>						
Skin 4	2000	0	100	65	380	130
Skin 5	2000	0	16	50	365	265
Skin 6	2000	0	65	65	415	330
Mean ± SD		0	122 ± 87	55 ± 8	252 ± 166	188 ± 127
Exsheathment	6.1%		2.8%	12.6%	9.4%	615 ± 206
						30.4%

removing the sieve along with the lung pieces, the medium was examined under a Carl-Zeiss stereo dissection microscope and larvae were collected with a finely-drawn pasteur pipette. The number of exsheathed NaL_3 recovered from the lungs of each animal were counted. NaL_4 and pre-adults were recovered from the small intestines on day 12 and 27 post-infection, respectively. The intestines were opened lengthwise and chopped into pieces of about 4–5 cm in petridishes (9 cm diameter) containing about 20 ml tissue culture medium. They were subsequently incubated at 37° C for 3–4 h. Many *N. americanus* larvae dislodged themselves from the intestine during incubation and were collected. Further the intestine pieces were scraped and all larvae collected. The number of larvae (NaL_4 and pre-adults) recovered from the intestine of each animal was counted. In some experiments, pre-adult *N. americanus* were collected from intestines 37 days after infection. The adult *N. americanus* were collected from the intestines 40 days after infection.

Statistical analysis. The development of *N. americanus* in young adult hamsters was compared with that of baby hamsters. The tabulated data were analysed wherever necessary using the Mann-Whitney U-test.

Results

*Comparative development of *N. americanus* in baby and young adult hamsters*

Results in Table 1 show that about 62% $EnNaL_3$ develop in baby hamsters compared to as few as 3% in young adults. About 40% of infected babies died and all surviving ones carried significantly higher worm burdens. Compared to baby hamsters, 80% of the young adults carried significantly lower worm burdens.

Infection of young adult hamsters with $ExNaL_3$

The inability of $EnNaL_3$ to penetrate the skin could be one reason for a lower worm burden in young adult hamsters. We felt that the infection rate could be increased if the skin is by-passed. $EnNaL_3$ exsheath while penetrating the skin. Our intention was to obtain such larvae freed from the sheath so that they could be injected parenterally. A method was developed, and $EnNaL_3$ were exsheathed. An average of about 31% $EnNaL_3$ were completely exsheathed and liberated into the external container (Table 2). They were designated exsheathed infective third stage larvae ($ExNaL_3$) and used for infection.

The numbers of *N. americanus* recovered from the intestines of young adult hamsters following infection with different doses as well as different routes are shown in Table 3. *N. americanus* did not establish well even after exsheathing and injecting parenterally. The worm burden remained less than 10 per hamster, and the percentage of infectivity varied significantly. Higher infection doses also did not improve the establishment of the parasite. Three- and 6-week-old hamsters carried similar worm burdens (Table 3).

*Monitoring the migration of *N. americanus* in lungs and intestines of baby and young adult hamsters*

Migration of different developmental stages of *N. americanus* was monitored in baby and young adult hamsters to pinpoint the possible factor(s) con-

Table 3. Development of *Necator americanus* in naive young adult hamsters exposed to ExNaL₃

Age of hamsters on the day of infection	Infection dose No. of ExNaL ₃ per animal	Route of infection*	No. of hamsters found infected/ No. exposed	Infection %	No. of <i>N. americanus</i> collected from small intestine of hamsters 40 days after infection	Individual recovery	Mean ± SD
<i>Experiment 1</i>							
3 weeks							
	50a	SC	1/5	20	2		0.4 ± 0.8
	50b**	SC	4/6	67	1, 1, 2, 2		1.0 ± 0.9
	50c	IP	5/10	50	1, 1, 1, 2, 2		0.8 ± 0.8
	50d	IT	0/6	0	none		0
6 weeks							
	50a	SC	5/5	100	1, 2, 3, 4, 8		3.6 ± 2.7
	50b**	SC	2/5	40	1, 2		0.6 ± 0.6
	50c	IP	0/5	0	none		0
	50d	IT	1/5	20	1		0.2 ± 0.4
<i>Experiment 2</i>							
3 weeks							
	100	SC	4/9	44	1, 2, 3, 5		1.4 ± 1.8
	500	SC	4/14	28	1, 4, 4, 5		1.0 ± 1.8
	2000	SC	3/3	100	1, 4, 6		3.7 ± 2.5

* SC = subcutaneous; IP = intraperitoneal; IT = intrathoracic

** Equal numbers of hamsters were dosed orally with ExNaL₃, but none developed a, b, c, d: Experiments were conducted at the same time with identical batches of ExNaL₃

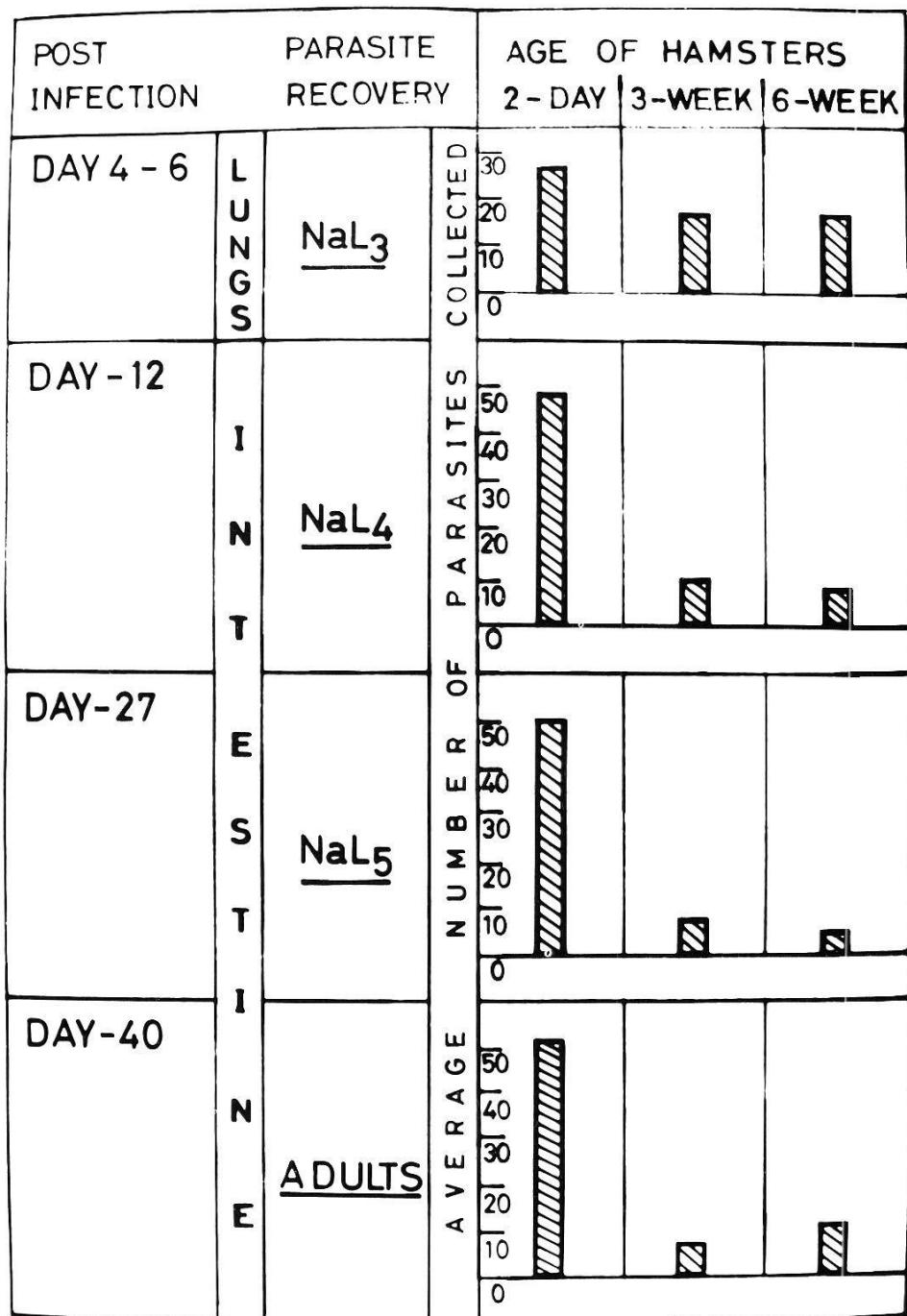


Fig. 1. Recovery of different developmental stages of *N. americanus* from baby and young adult hamsters at different intervals after infection with 100 EnNaL₃ percutaneously. Bars represent the average number of parasites collected at each point. The worm burdens of young adult (3- and 6-week-old) groups were not statistically different from baby hamsters in lungs ($P > 0.05$) but in the intestines young adults carried significantly lower worm burden ($P < 0.05$) than that of baby hamsters during all intervals.

trolling their development in adult hamsters. In a preliminary experiment, we recovered *in vivo* exsheathed NaL₃ from the lungs 4 to 6 days after infection. NaL₄ and pre-adult stages were collected from intestines 12 and 27 days after infection, respectively. Adult (male and female) *N. americanus* were collected

from the intestine 40 days after infection. Two sets of experiments were conducted. In each set 10 hamsters of each age group (2-day-, 3- and 6-week-old) were used and each received 100 EnNaL₃ percutaneously. Two animals from each age-group were sacrificed on day 4, 6, 12, 27 and 40 PI. Their lungs and intestines were incubated and *N. americanus* were recovered. No parasites were recovered from lungs 12 days after infection. Intestines were free from parasites up to 6 days PI. This observation was uniform in all hamsters irrespective of their age group. The data from 4 animals at each time point were pooled and shown in Fig. 1. For convenience, parasites recovered from lungs on 4 and 6 days PI (n = 8) were pooled and the data are shown in Fig. 1. In lungs, about 17% of larvae were recovered from young adults as opposed to about 27% in babies. This was not statistically significant (P > 0.05). However, young adults (n = 4) carried significantly lower worm burdens in the intestines 12, 27 and 40 days after infection (P < 0.05 at all time points) than baby hamsters. Thus, a significant difference between young adults and baby hamsters was found only for worm burdens in the intestines.

Discussion

As judged from the worm burden in the intestines, only 3% of NaL₃ developed in young adult hamsters whereas in babies, the development was as high as 60%. From these results, it is apparent that young adults are resistant to the establishment of *N. americanus*. In order to assess the possibility of using young adults as experimental hosts for immunological studies the nature of age resistance needs to be understood. The main objective was to induce higher worm burdens in adults. It was thought that the skin may act as a barrier and therefore it was by-passed. Moreover, in vitro exsheathed larvae were used for infection. Whatever the parenteral route adopted for infection, there was no improvement in the establishment of *N. americanus*. Even higher infection doses did not improve the worm burden. It could be argued that in vitro exsheathment might have affected the larval infectivity. Baby hamsters were infected with ExNaL₃ prepared from young adult hamster skin. Six days after infection, lungs of infected babies were examined and an average of 24% larvae were recovered (unpublished observation). We have evidence to show that ExNaL₃ are infective even after passing through the hamster skin.

At this stage, it is reasonable to inquire into the fate of injected larvae, and the inability of greater numbers of ExNaL₃ to reach maturity in young adults. It is possible that larvae might have been killed en route to the intestine. To prove this either histopathology or larval monitoring could be performed. Applying the latter we found that in the lungs, babies as well as young adults had similar worm burdens. In the intestines, however significant differences were seen in the worm burdens; young adults carried about 10 worms each compared to about 50 worms in baby hamsters.

Examination of the migration of *N. americanus* from the lungs into the intestine revealed that young adult hamsters expelled the majority of the parasites from the intestine accounting for the lowered worm burden observed. Furthermore, the lungs of babies as well as young adults were free from parasites 12 days after infection which substantiates the worm expulsion from intestines of young adults.

Comparative monitoring of worms indicated the intestine as the site of resistance. However, the nature and the mechanisms of resistance against the establishment of *N. americanus* in young adults are not yet clearly demonstrated. Kiyono et al. (1983) showed the immunological involvement of the gut-associated lymphoreticular tissues. The role played by mucosal mast cells and biogenic amines in the expulsion of gut nematodes is known. Moreover, in aged hamsters, a high level of IgA (Smith et al., 1983) and potent killer cell activities (Dutta et al., 1979; Haddada et al., 1980) have been reported. It will be relevant to pinpoint which of the resistant factors are directed against *N. americanus* and whether they are similar to the ones mediating the spontaneous expulsion of *Nippostrongylus brasiliensis* (Miller and Nawa, 1979; Levy and Frondoza, 1983) and other helminths (Castro, 1981).

Acknowledgments

Thanks are extended to Dr. Sheila Talwalker of this Centre for statistical analysis.

Castro G. A.: Physiology of the gastrointestinal tract in the parasitized host. In: Physiology of the gastrointestinal tract, ed. by L. R. Johnson, p. 1381–1406. Raven Press, New York 1981.

Dutta S. K., Gallagher M. T., Trentin J. J.: Natural cell-mediated cytotoxicity in hamsters. *Int. J. Cancer* 23, 728–734 (1979).

Haddada H., Duthu A., Vaux-Saint-Cyr Ch. De: Natural cytotoxic activity in different experimental conditions in hamsters. *Ann. Immunol.* 131, 187–198 (1980).

Kiyono H., Mosteller L. M., Eldridge J. H., MiChalek S. M., McGhee J. R.: IgA responses in XID mice: oral antigen primes Peyer's patch cells for in vitro immune responses and secretory antibody production. *J. Immunol.* 131, 2616–2622 (1983).

Levy D. A., Frondoza C.: Immunity to intestinal parasites: role of mast cells and goblet cells. *Fed. Proc.* 42, 1750–1755 (1983).

Miller H. R. P., Nawa Y.: Immune regulation of intestinal goblet cell differentiation, specific induction of non-specific protection against helminths. *Nouv. Rev. franç. Hémat.* 21, 31–45 (1979).

Ogilvie B. M., McLaren D. J., Worms M. J.: Studies with *Necator americanus* maintained in hamsters. *Nuc. Tech. Helm. Res. IAEA Vienna* p. 55–60. Vienna 1975.

Rajasekariah G. R., Dhage K. R., Deb B. N., Bose S.: *Necator americanus* and *Ancylostoma ceylanicum*: development of protocols for dual infection in hamsters. *Acta trop. (Basel)* 42, 45–54 (1985).

Sen H. G., Seth D.: Complete development of the human hookworm, *Necator americanus*, in golden hamsters, *Mesocricetus auratus*. *Nature (Lond.)* 214, 609–610 (1967).

Smith D. J., Ebersole J. L., Taubman M. A.: Local and systemic immune response in aged hamsters. *Immunology* 50, 407–413 (1983).