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EVIDENCES AGAINST A SIGNIFICANT ROLE OF *MUS MUSCULUS* AS NATURAL HOST FOR *ANGIOSTRONGYLUS COSTARICENSIS*

Fernanda Teixeira dos SANTOS, Viviane M. PINTO & Carlos GRAEFF-TEIXEIRA

SUMMARY

Wild rodents have been described as the most important hosts for *Angiostrongylus costaricensis* in Central America and southern Brazil. Sinantropic rodents apparently do not play a significant role as natural hosts. A search for natural infection failed to document worms in 14 mice captured in the house of a patient with diagnosis of abdominal angiostrongylosis and experimental infection of a "wild" *Mus musculus* strain and groups of albino Swiss mice were carried out. Mortality was not significantly different and varied from 42% to 80% for Swiss mice and from 26% to 80% for "wild" mice. The high mortality of a "wild" *M. musculus* infected with *A. costaricensis* was very similar to what is observed with most laboratory mice strains. These data may be taken as indications that *M. musculus* is not a well adapted host for *A. costaricensis*, although susceptibility was apparently higher with "wild" populations of *M. musculus* as compared to Swiss strain.

KEYWORDS: *Angiostrongylus costaricensis; Mus musculus;* Angiostrongylosis.

INTRODUCTION

Angiostrongylus costaricensis (*A. costaricensis*) Morera e Céspedes, 1971 is a parasitic nematode living inside branches of the mesenteric artery in rodents. Terrestrial molluscs are the intermediate hosts, shedding infective third stage larvae (L3) with their mucous secretions[11]. Accidental human infection has been reported in the Americas, from Mexico[17] to Argentina[3]. In southern Brazil the infection appears to be quite frequent and severe clinical disease have been sporadically detected[1, 2, 9].

The most important definitive host in Central America is the cotton rat, *Sigmodon hispidus*[10], with geographic distribution limited from southern United States to northern Peru and Venezuela[14]. In southern Brazil, *Oryzomys nigripes* and *Oryzomys ratticeps* were identified as natural definitive hosts[5].

The domestic mouse, *Mus musculus* (*M. musculus*) and the rat, *Rattus norvergicus*, were never incriminated as important hosts for *A. costaricensis*[5, 12, 15]. High morbidity and mortality is known to occur with experimental infection in laboratory strains of *M. musculus* and by extension this fact has been considered as a partial explanation for the absence of naturally infected mice in endemic areas[8, 12]. The objective of the present experiments was to describe mortality in *M. musculus* infected with *A. costaricensis*, comparing a "wild" strain with the Swiss albino strain. Results from a search for natural infection in a rural site in Rio Grande do Sul (RGS) are also reported.

MATERIAL AND METHODS

Search for natural infection – *M. musculus* and *Rattus rattus* (*R. rattus*) were captured during a "seasonal cleaning" of a crops' storehouse in the rural area of Arvorezinha, RS (Macegal, 28° 45' S; 52° W) at the same site of a previous study[4]. The strategy was suggested by local peasants and consisted in removing the remainings of seasonal corn stores. Mice tried to escape and were captured, immediately

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Laboratório de Parasitologia, Dept. Ciências Microbiológicas e Curso de Pós-Graduação em Biociências (Zoologia), Instituto de Biociências, PUCRS, Porto Alegre, RS, Brazil.
Correspondence to: Dr. C. Graeff-Teixeira, Rua Marquês do Pombal 799/801. 90540-010 Porto Alegre, RS, Brazil.

sacrificed and examined for the presence of worms in the branches of mesenteric artery.

Establishment of the "wild-RGS" strain – Domestic mice were captured at four different locations, in Rio Grande do Sul (Brazil's southernmost State): a crop research facility (Estação Experimental da Secretaria de Agricultura, Viamão), houses in Passo Fundo and Cidreira, and a bakery in Porto Alegre. A colony was established in the laboratory and the strain named RGS. Only F1 and subsequent generations were used in the experiments.

Source of the parasite – The "Santa Rosa" strain of *A. costaricensis* was used for the experimental infections and has been maintained since 1992 in the laboratory, by passages through Swiss mice and veronicelid terrestrial slugs, *Phyllocaulis soleiformis*.

Experimental infection – Six goups of RGS mice with a number of animals ranging from 9 to 23 (Table 1) were gently anesthesized with inhaled ether and inoculated "per os" with 8 L3 per animal. The number of animals in each group varied according to the available number of L3. Mortality was recorded at 4th week post-infection (PI) and the number of worms was determined by dissection of the mesenteric arterial system. The same observations were done in five groups of Swiss mice infected as part of the procedures for mantainance of the cycle in the laboratory.

RESULTS

A. costaricensis was not isolated from 14 *M. musculus* and 4 *R. rattus rattus* that were captured and examined in Macegal, RS.

In experimental infections, mortality varied from 26% to 80% (average of 54% – variance = 394) for RGS mice (Table 1) and from 42% to 80% for Swiss mice (average of 58% – variance = 251) (Table 2). Mortality in non-infected (negative control) RGS mice and Swiss mice was always zero. The difference in the avarage mortlity of infected Swiss and RGS mice is not statistically significant as indicated by variance analysis at p = 0.05, resulting in a F value of 1.04, lower than the critical F value of 5.05.

All Swiss mice that survived were either free from infection (18/27) at the time of examination or had a unisexual infection (9/27) (Tables 2 and 3). Bisexual infection was documented in 20/35 RGS mice and unisexual infection in 12/35. Only 3/35 were not infected (Tables 1 and 3). A total of 104 worms (45 males and 59 females) from RGS mice and 11 (3 males and 8 females) from Swiss mice were recovered and the ratio of recovered worms per infected survivor mice was 1.22 for Swiss and 3.25 for RGS mice (Table 3).

DISCUSSION

Most of the rodent species found infected in Central America and southern Brasil are wild rodents: *Tylomys watsoni, Liomys salvini, L. adspersus, Proechimys semispinosus, Peromyscus nudipes, Zygodontomys microtinus, Oryzomys albigularis, O. caliginosus, O. fulvescens, O. nigripes* and *O. Ratticeps*[5, 12, 15]. The cotton rat, *Sigmodon hispidus* is found in the houses and peridomiciliar areas[12]. Among the other well recognized sinantropic rodents, the prevalence in *R. rattus* may be high (20% in Costa Rica) or low (4% in Panamá), only 2 out of 94 *R. norvergicus* (2,1%) had a documented infection in Costa Rica and *M. musculus* has not been found

TABLE 1

Outcome of experimental infection of a "wild" strain (RGS) of *Mus musculus* with *Angiostrongylus costaricensis*: mortality and number of worms recovered from mice surviving at 4 weeks post-inoculation.

Group	Mortality deaths/animals (%)	Number of worms recovered from individual mouse		Totals
RGS 1	4/9 (44)	counting not done		–
RGS 2	17/21 (80)	counting not done		–
RGS 3a	6/23 (26)	males	0 1 2 2 0 1 1 0 2 0 1 1 1 1 1 0 1	15 males
		females	2 1 1 0 4 0 3 2 1 3 3 1 1 0 1 0 0	23 females
		total	2 2 3 2 4 1 4 2 3 3 4 2 2 1 2 0 1	38 total
RGS 3b	5/14 (36)	males	3 1 2 2 3 4 1 5 0	21 males
		females	2 1 0 4 1 1 1 1 2	13 females
		total	5 2 2 6 4 5 2 6 2	34 total
RGS 4	12/17 (70)	males	2 0 1 0 0	03 males
		females	4 1 0 0 0	05 females
		total	6 1 1 0 0	08 total
RGS 5	9/13 (69)	males	1 3 1 1	06 males
		females	5 6 2 5	18 females
		total	6 9 3 6	24 total

TABLE 2

Outcome of experimental infection of Swiss strain of *Mus musculus*, with *Angiostrongylus costaricensis*: mortality and number of worms recovered from mice surviving at different time post-inoculation.

Group	Time (weeks)	Mortality deaths/animals (%)	Number of worms recovered from individual surviving mouse		Totals
G8B	17	12/15 (80)	males	0 0 0	0 male
			females	0 0 0	0 female
			total	0 0 0	0 total
G9B	16	4/9 (44)	males	0 0 0 0 0	0 male
			females	0 0 0 0 1	1 female
			total	0 0 0 0 1	1 total
G9A	16	5/12 (42)	males	0 0 0 0 0 0 0	0 male
			females	0 0 0 0 0 0 1	1 female
			total	0 0 0 0 0 0 1	1 total
G10B	22	6/12 (50)	males	0 0 0 2 0 0	2 males
			females	0 0 1 0 2 1	4 females
			total	0 0 1 2 2 1	6 total
G11A	31	17/23 (74)	males	0 0 0 0 1 0	1 male
			females	0 0 0 1 0 1	2 females
			total	0 0 0 1 1 1	3 total

TABLE 3

Experimental infection of two strains of *Mus musculus: Swiss and a "wild" RGS strain.* Mortality and analysis of survivor mice: number of non-infected, unisexually and bisexually infected animals. The ratio between total worms recovered and the number of infected mice was calculated from the data presented in Tables 1 and 2.

Strain	Avarage mortality	Number of non-infected mice	Mice with unisexual infection	Mice with bisexual infection	Ratio total worms/infected mice
SWISS	58%	18/27 (66.6%)	9/27 (33.3%)	0/27	1.22 (11/9)
RGS	54%	3/35 (8.5%)	12/35 (34.2%)	20/35 (57.1%)	3.25 (104/32)

infected[12, 15]. The locality of Macegal, in southern Brazil, was studied in 1988, and the lack of infection in the two occurring sinantropic rodent species *M. musculus* and *R. rattus rattus* was confirmed in the search now reported. In the study of TESH et al. (1973)[15] *R. rattus* was not identified at sub-species level, but there is a possibility that looking for less sinantropic sub-species, like *Rattus rattus alexandrinus* or *Rattus rattus frugivorus*, the natural infection may be documented with higher prevalences.

Sinantropic rodents are not easily trapped, probably because their habitat is plenty of food sources, especially in rural areas in northern Rio Grande do Sul (RGS). For this reason we returned to the site of a previous search for definitive hosts and tried to capture *M. musculus* with a strategy suggested by local peasants and examination of mesenteric vessels did not reveal mice infected with *A. costaricensis*. Living inside houses, mice probably expose themselves less intensively to the infective forms of a parasite of wild rodents and molluscs. In several rural areas of RGS, sinantropic molluscs of Veronicellidae and Limacidae families were virtually absent and a giant land snail from the genus *Megalobulimus* is the suspected wild natural host for *A. costaricensis*[6].

Concerning the domestic mouse, the infection of Swiss strain in the laboratory results in high mortality, what has been taken as an explanation for the lack of detection of natural infections[12]. Inbred strains (BALB/c, DBA/2 and CH3/He) also present with mortality higher than 50% at 4 weeks post-infection, with the interesting exception of C57BL/6[8]. The high mortality (54%) in experimentally infected "wild-RGS" outbred strain, taken as more representative of natural populations of *M. musculus* than laboratory strains, could be an indication that this species does not play a significant role as a natural host for *A. costaricensis*.

The study of experimentally infected mice demonstrated bisexual infection in 57.1% (20/35) of RGS mice and failed to demonstrate it in Swiss mice groups. Only mice harboring at least a male/female couple may contribute for mantainance of the parasite, and these experimental data suggest a higher susceptibility of wild RGS mice to infection

as compared to the Swiss laboratory strain. This is clearly seen with a different arrangement of data (Table 3) and the average number of worms produced per mice was calculated to be 1.22 (11/9) for Swiss mice and 3.25 (104/32) for RGS mice.

The finding of a female worm alive at 31st week PI is the highest observed longevity for this nematode, in the infection of *M. musculus*. Contrary to what is known to happen with infections in a natural host like *O. nigripes*, study of longevity of worms infecting Swiss mice is hampered by the host's mortality.

Studies of mortality and morbidity may not be sufficient to evaluate the degree of host-parasite adaptation and the consequent contribution of a given host-species to the survival of a parasite. More quantitative approaches in experimental models and extensive ecological investigations are required for a better evaluation of host-parasite coevolutionary status[4]. Anyway, alive but ill mice may be easily killed by predators and that may be the case with *M. musculus* in natural conditions. Informations obtained experimentally in the *M. musculus* model should always consider the possibility that this murine rodent is not well-adapted host for *A. costaricensis*[13].

In conclusion, the course of infection with *A. costaricensis* in a "wild" RGS strain was found to be different from the observed with Swiss albino mice. Although RGS strain appears to be more susceptible and to survive with more frequent bisexual infections, the lack of identification of natural infections and the high mortality in experimental conditions as shown in the present report, further support the hypothesis claiming an insignificant contribution of *M. musculus* for the cycle of *A. costaricensis*.

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RESUMO

Evidências contrárias a um papel significativo de *Mus musculus* como hospedeiro natural do *Angiostrongylus costaricensis*.

Roedores silvestres têm sido descritos como os hospedeiros mais importantes do *Angiostrongylus costaricensis* na América Central e no Brasil. Roedores sinantrópicos aparentemente não desempenham um papel significativo como hospedeiros naturais. Relata-se a pesquisa negativa para infecção em 14 camundongos capturados na casa de um doente com diagnóstico de angiostrongilose abdominal e dados da infecção experimental de animais de uma cepa "natural" de *Mus musculus* e de camundongos albinos Swiss. Não houve diferença significativa de mortalidade, que variou entre 42% e 80% para a cepa Swiss e 26% e 80% para a cepa "natural" (RGS). A alta mortalidade dos camundongos RGS infectados com *A. costaricensis* foi semelhante ao que é observado com várias cepas de laboratório. Estes dados podem ser tomados como indicações de que *Mus musculus* não é hospedeiro bem adaptado ao *A. costaricensis*, embora a susceptibilidade de camundongos "naturais" pareça ser maior, quando comparados com a cepa Swiss.

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