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Influence of Rat Strain on Larval Production by the Parasitic Nematode *Strongyloides ratti*

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ABSTRACT: The course of infection with *Strongyloides ratti* in a range of rat strains was assessed by monitoring the production of larvae. To our knowledge, this is the first such study of *S. ratti* using its natural host *Rattus norvegicus*. Host strain influenced the pattern of larval production. The results were qualitatively the same for 2 *S. ratti* lines of North American and Japanese origin.

Nematodes in the genus *Strongyloides* are important gastrointestinal parasites of humans and domestic livestock (Dawkins, 1989). *Strongyloides ratti* is a parasite of rats and is used extensively as a convenient laboratory model of strongyloidiasis. The influence of host genetics on the course and kinetics of infection has received little attention in *S. ratti*. This is despite its possible significance in various aspects of parasitic disease in general (Grenfell and Dobson, 1995). With *S. ratti*, the majority of work in this area has been conducted using mice (*Mus musculus*) in which a number of inbred and mutant strains have been shown to differ in susceptibility to infection with this nematode (e.g., Dawkins et al., 1980; Nawa et al., 1985, 1988). Studies with the natural host (*Rattus norvegicus*) have been confined to examination of the effects of gender (e.g., Katz, 1967) and a single immunologic mutation (Gemmill et al., 1997). Our aim here was to quantify the pattern of larval production by *S. ratti* in various inbred and random bred strains of the natural host *R. norvegicus*. The rat strains used were chosen on the basis of differing profiles of antibody production in response to another gastrointestinal nematode, *Nippostrongylus brasiliensis* (Kennedy et al., 1990), and differ in haplotype at a major histocompatibility complex (MHC) (RT1) locus.

We carried out 2 experiments. In the first we compared larval production by a single *S. ratti* line (ED5 Heterogonic; Viney, 1996; referred to here as ED5) in a random bred rat strain (Wistar) and 4 inbred strains. The second was designed to provide greater detail on patterns of larval production in 2 rat strains that differed consistently in the first experiment. Specifically, we sampled infections more frequently and used larger numbers of rats. In addition, in this second experiment, we examined whether the same patterns were observed for a second *S. ratti* line (ED279) of different geographic origin. ED5 and ED279 are isofemale lines descended from North American and Japanese isolates, respectively (Viney, 1996: ED279 derives from isofemale line 132).

Methodology was the same in both experiments. Six-week-old male rats (Harlan UK) were used with food and water provided ad libitum. Parasite lines were maintained by serial passage in laboratory rats. Experimental animals were infected by subcutaneous injection with 500 infective third-stage larvae

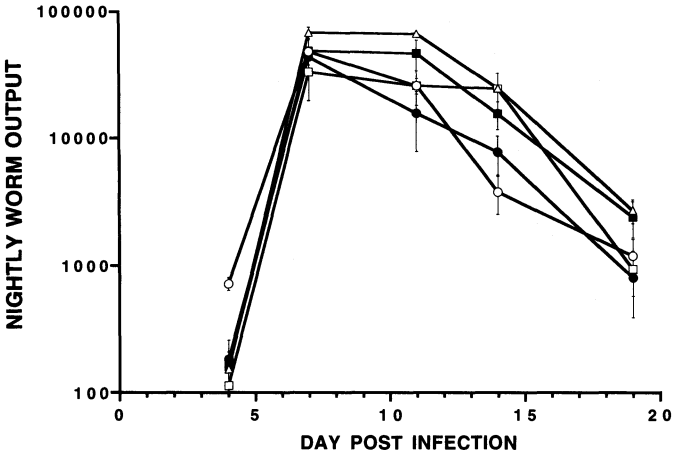

FIGURE 1. Nightly worm output from 5 strains of rat infected with 500 iL3 of the *Strongyloides ratti* line ED5. For each rat strain, haplotype at the MHC RT1 locus is given in parentheses following the sample size. Open triangles = Wistar (n = 2, variable); open circles = PVG (n = 4, $RT1^c$); open squares = LOU (n = 4, $RT1^u$); closed circles = F344 (n = 4, $RT1^{lv1}$); closed squares = LEW (n = 4, $RT1^l$). Errors are ±1 SE. In some cases, error bars are smaller than the symbol.

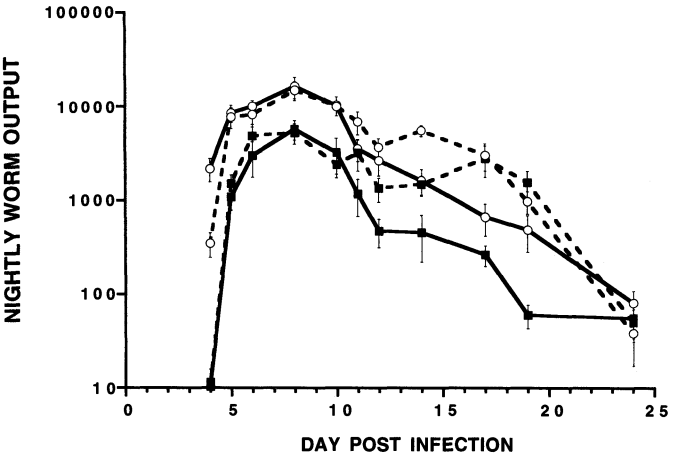

FIGURE 2. Nightly worm output from PVG and Wistar rats infected with 500 iL3 of the *Strongyloides ratti* lines ED5 or ED279. Open circles, broken line = Wistars infected with ED5 (n = 5); closed squares, broken line = Wistars infected with ED279 (n = 5); open circles, solid line = PVGs infected with ED5 (n = 6); closed squares, solid line = PVGs infected with ED279 (n = 6). Errors are ±1 SE. In some cases, error bars are smaller than the symbol.

(iL3) and the numbers of parasite offspring emerging from feces (nightly worm output) monitored throughout infection. Feces were collected onto damp paper overnight, cultured in glass petri dishes, and incubated for 2 days at 25 C or 3 days at 19 C. Cultures were then washed extensively in distilled water to collect worms into a known volume and the numbers present determined by counting worms in repeated samples under a binocular microscope. The positions of animals in the collecting apparatus, the order in which cultures were made and subsequently processed, as well as the positions of cultures in incubators were randomized anew at each sampling point. The total number of parasite offspring produced over the course of infection (total worm output) was estimated by numerical integration under the worm output by time curves. Data were analyzed by conventional analysis of variance (ANOVA).

Nightly worm outputs from the the first experiment are shown in Figure 1. Total worm output was highest in Wistar rats and lowest in F344 rats. One-way ANOVA using Bonferroni/Dunn multiple means comparison revealed that total worm output was higher in Wistar rats than in PVG and F344 rats (Wistar × PVG, $P = 0.049$; Wistar × F344, $P = 0.03$). The same comparison yielded P-values of 0.07 and 0.22 for the LOU and LEW strains, respectively.

In our second experiment, we examined patterns of larval production in Wistar and PVG rats with 2 lines of *S. ratti*. Nightly worm outputs from the 4 groups are shown in Figure 2. Two-way ANOVA on total worm ouput revealed the following. Total worm ouput was not significantly greater in Wistar rats than in PVG rats ($F_{1,18} = 3.1$, NS). Infection with ED279 led to lower total worm output regardless of rat strain ($F_{1,18} = 42.2$, $P < 0.0001$; worm line × rat strain interaction $F_{1,18} = 0.047$, NS).

At first sight, the results of the 2 experiments appear contradictory. In the first experiment, there was a significant difference in total worm output between PVG and Wistar rats infected with ED5. In the second, there was not. By analyzing the early and late parts of our second experiment separately, the reason for this inconsistency becomes clearer. Nightly worm output from rats infected with ED5 was highest in PVG rats prior to day 11 postinfection (PI) and highest in Wistar rats thereafter. Total worm output until and including day 10 PI did not differ significantly between rat strains ($F_{1,9} = 0.3$, NS), but total worm output from day 11 PI onward did ($F_{1,9} = 12.6$, $P < 0.01$). This difference is given undue weight in the first experiment. In the first experiment, only 2 time points were sampled prior to day 11 PI, the period when nightly worm output was at its highest. The difference in the timing of sampling leads to an underestimate of total worm output prior to day 11 PI and to the (false) conclusion that total worm output differs significantly between the PVG and Wistar strains. Furthermore, if only those sampling points common to both experiments are used to calculate total worm output in the second experiment, the effect of rat strain becomes significant for infections with both ED5 and ED279 ($F_{1,18} = 12.17$, $P < 0.01$; worm line × rat strain interaction $F_{1,18} = 0.51$, NS).

In the second experiment, there is also a difference in the pattern of worm ouput in the latter period. This is apparent as an interaction of rat strain × day PI in a repeated-measures ANOVA ($F_{5,45} = 2.55$, $P = 0.041$). Thus, from day 11 PI on, ED5 behaved differently in PVG and Wistar rats without significantly affecting total worm output across the experiment as a whole.

Our results illustrate 2 points. First, rat strain had no overall effect on total worm output. Second, rat strain did have subtle effects on the pattern of larval production. The observed magnitude of these effects depends crucially on when and how often infections are sampled. This observation is not surprising but is nevertheless important in quantitative studies of host–parasite interactions. Much work in this area relies on measurements taken at a fraction of possible sampling points (e.g., examine relevant figures in Wakelin and Blackwell [1988], chapters 4 and 5).

The fact that patterns of larval production by *S. ratti* differ between rat strains is a novel finding in this host–parasite sys-

tem. These differences may stem from divergent aspects of immunology and parasite reproductive strategies in different host strains. The overall difference in total worm output between ED5 and ED279 could be the result of environmental variance or it may have a genetic cause.

In summary, we report that genetic variation in the host (*R. norvegicus)* leads to subtle alteration in patterns of larval production by *S. ratti.* In addition, our results caution that experiments employing incomplete sampling regimes can fail to determine accurately the outcome of host–parasite interactions. Certainly, much remains to be clarified concerning the host–parasite relationship in the system studied here.

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Helminths of Six Species of *Anolis* Lizards (Polychrotidae) from Hispaniola, West Indies

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ABSTRACT: From Hispaniola, an island in the West Indies, 6 species of anoles, *Anolis cristatellus, Anolis etheridgei, Anolis eugenegrahami, Anolis hendersoni, Anolis monticola,* and *Anolis olssoni* were examined for helminths. Helminths representing 2 species of trematodes, *Mesocoelium monas* and *Urotrema scabridum;* 1 species of cestode, *Oochoristica maccoyi*; 11 species of nematodes, *Atractis scelopori, Oswaldocruzia lenteixeirai, Parapharyngodon cubensis, Skrjabinoptera leiocephalorum, Trichospirura teixeirai, Ascarops* sp., *Physocephalus* sp., *Porrocaecum* sp., *Rhabdias* sp., Acuariidae gen. sp. and Cosmocercidae gen. sp.; and 1 species of acanthocephalan, *Centrorhynchus* sp., were found. *Skrjabinoptera leiocephalorum, Ascarops* sp., and *Physocephalus* sp. from *A. cristatellus* and all helminths recovered from the other 5 anole species represent new host records.

Seven of 41 species of *Anolis* on the island of Hispaniola in the West Indies (see Schwartz and Henderson, 1991), *Anolis armouri, Anolis bahorucoensis, Anolis barahonae, Anolis brevirostris, Anolis chlorocyanus, Anolis coelestinus* and *Anolis cybotes,* have been reported to harbor helminths (Fobes et al., 1992; Moster et al., 1992; Lenart et al., 1994; Goldberg, Bursey, and Cheam, 1996). The purpose of the present study is to report helminths from 6 additional species from Hispaniola; *Anolis cristatellus, Anolis etheridgei, Anolis eugenegrahami, Anolis hendersoni, Anolis monticola,* and *Anolis olssoni.* Information on the ecology of these anoles is summarized in Schwartz and Henderson (1991).

Anoles (n = 134) were borrowed from the herpetology collection of the Bobby Witcher Memorial Collection, Avila College (BWMC) and the University of Kansas Natural History Museum (KU): *A. cristatellus,* n = 34, collected 1997, La Romana Province, Dominican Republic (BWMC 06078–06111); *A. etheridgei,* n = 20, 1971, Monseñor Nouel Province, Dominican Republic (KU 271040–271059); *A. eugenegrahami,* n = 20, 1978, Dept. l'Ouest, Haiti (KU 270706–270711, 270713–270726); *A. hendersoni,* n = 20, 1977, 1979, Dept. l'Ouest, Haiti (KU 270136, 270142–270145, 270146–270152, 270155, 270159, 270162, 270181, 270183, 270188–170190); *A. monticola,* n = 20, 1979, Dept. du Sud, Haiti (KU 259788–259807); *A. olssoni,* n = 20 (all from the Dominican Republic) 1971, Azua Province (KU 259350, 259352), 1963, 1971, 1974, Barahona Province (KU 259356–259358, 259360–259362, 259366, 259368), 1969, 1972, Independencia Province (KU 259371, 259374, 259380, 259383–259384), 1963, Peravia Province (KU 259390, 259392), 1969, 1971, San Juan Province (KU 259397–259398, 259400). Snout–vent length (SVL) and range in mm for each lizard species are given in Table I.

The body cavity was opened by a longitudinal incision from throat to vent and the gastrointestinal tract was removed by cutting across the anterior esophagus and the rectum. Esophagus, stomach, small intestine, large intestine, gall bladder, liver,