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EFFECTS OF HANTAVIRAL INFECTION ON SURVIVAL, GROWTH AND FERTILITY IN WILD RAT (*RATTUS NORVEGICUS*) POPULATIONS OF BALTIMORE, MARYLAND

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ABSTRACT: Survival, growth rates, body size and fertility of wild caught Norway rats (*Rattus norvegicus*), infected and uninfected with a *Hantavirus* (antigenically related to Seoul virus), were compared. No differences were found in the survival of seronegative versus seropositive rats, as measured by mark-recapture experiments. Growth rates, as measured by weight gain but not by increased body length, were slower in seropositive, sexually mature (>200 g) rats, although no differences in the ultimate body size of infected versus uninfected rats were found. No differences in external measures of sexual maturity, or in embryo counts or testes sizes, were found for infected versus uninfected rats. We conclude that hantaviral infections have little or no impact on demographic processes in Norway rat populations.

Key words: Norway rats, *Rattus norvegicus*, *Hantavirus*, Seoul virus, survival, growth, fertility, host-virus relationship, zoonotic disease.

INTRODUCTION

The genus *Hantavirus*, of the family Bunyaviridae, was defined to include viruses responsible for human diseases collectively termed hemorrhagic fever with renal syndrome (HFRS; Schmaljohn et al., 1985). These viruses are primarily maintained in rodents of the superfamily Murioidea (for review see Yanagihara and Gadjusek, 1987), and currently four well documented ecological groupings exist: Hantaan virus with *Apodemus agrarius* (Lee et al., 1978); Seoul virus with *Rattus norvegicus* (Lee et al., 1982); Puumala virus with *Clethrionomys glareolus* (Brummer-Korvenkontio et al., 1980); and Prospect Hill virus with *Microtus pennsylvanicus* (Lee et al., 1985).

Different hantaviruses experimentally inoculated into weanling rodents, considered to be their primary hosts, caused subclinical infections with little or no resulting pathology (Lee et al., 1981; Yanagihara et al., 1985). Virus persisted in several tissues and was shed continually, or sporadically, in urine, feces and/or oropharyngeal secretions. Fluorescent and neutralizing an-

tibody circulated concurrently with viral shedding, and persisted after infectious virus was no longer demonstrable in tissues.

Field studies of Norway rats (*R. norvegicus*) in the USA (Childs et al., 1985, 1987a, b) and Japan (Arikawa et al., 1986), and on bank voles (*C. glareolus*) in Belgium (Verhagen et al., 1986), suggested most hantaviral infections were acquired in an age-dependent fashion, as rodents reach young adult to adult status. Transplacental transmission of hantaviruses was not demonstrated in experimental infections and/or in progeny from infected field caught *A. agrarius* (Lee et al., 1981), *C. glareolus* (Verhagen et al., 1986; Bogdanova et al., 1987), or *R. norvegicus* (Lloyd and Jones, 1987; G. W. Korch, pers. obs.). Perinatal transmission of virus in situations where dams are both seropositive and infectious may be unlikely, as passively acquired maternal antibody protected suckling-juvenile rats from viral challenge for periods up to 10 wk of age (Zhang et al., 1988).

Thus, the pattern of chronic hantaviral infection with persistent shedding of virus

has not been associated with any cost to experimental adult hosts, and field data suggest it is this older strata of the population which becomes infected. This situation is in striking contrast to the effects of some other viruses which also cause persistent infections in rodent hosts. For example, two arenaviruses, Machupo and Junin, primarily infect different species of rodents of the genus *Calomys* in Bolivia and Argentina, respectively. In these virus-host pairings, vertically or horizontally transmitted viruses can result in decreased survivorship and growth and/or increased fetal wastage in chronically infected adult females (Webb et al., 1975; Vitullo et al., 1987). These effects of viral infection on survival, growth and reproduction can obviously influence the fitness of an individual animal, as measured by life-long production of offspring, and, in the case of Machupo virus, have been proposed as regulatory mechanisms on the size and genetic structure of *Calomys callosus* populations (Johnson, 1985).

In earlier reports we focused on the isolation of a *Hantavirus* (Baltimore rat virus—antigenically related to Seoul virus) from Baltimore City Norway rats (Childs et al., 1987b), the characteristics of infected populations (Childs et al., 1987a, b), possible mechanisms of transmission (Glass et al., 1988a), and the ecology of the rodent host (Glass et al., 1988b, 1989). Here, we examine evidence from wild caught animals to determine if hantaviruses influence the demographic processes of rat populations by compromising survivorship, growth or fertility.

MATERIALS AND METHODS

Norway rats were trapped from 1980 to 1988 at 11 residential sites throughout Baltimore, Maryland (USA) using Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin 54487, USA). Rats captured from parklands are excluded from these analyses because our previous studies have documented significant differences in the size (body weight and length) and growth rates of residential versus parkland animals (Glass et al., 1988b, 1989). Detailed descriptions of the study areas, trap-

ping protocols and ecology of the rat populations have been provided elsewhere (Glass et al., 1989).

All captured rats were transported to the laboratory and anesthetized with inoculations of ketamine HCl and xylazine (10:1). Blood samples were collected by cardiac puncture, and data recorded on sex, external evidence of maturity (perforate vaginal orifices in females, scrotal testes in males), weight (to the nearest g) and body length (nose to anus, to the nearest mm). From 1980 to 1983 only weights were obtained, so in some analyses the sample sizes for weight and body length vary. A subsample (379; 58% of total individuals captured) of rats was sacrificed and data on number of embryos, and length and width of testes, were obtained for females and males, respectively. Testes volumes were computed using the formula of a prolate spheroid as described elsewhere (Glass et al., 1989). During the period from October 1984 to July 1986, at three sites, animals were marked with unique ear tags and released at their location of capture (mark-release-recapture (MRR) study). A minimum of 30 traps/site/night were set, and each site was trapped for three consecutive nights at 1 mo intervals. Rats were held in the laboratory for ≤ 12 hr between their capture and release (Childs et al., 1987a).

Indirect fluorescent antibody tests were performed using spot slides of Vero E-6 cells infected with prototype Hantaan virus, strain 76-118 (Salk Institute, Swiftwater, Pennsylvania 18370, USA) and FITC-conjugated goat anti-rat IgG (heavy and light chain specific; Cappel Laboratories, Westchester, Pennsylvania 19380, USA). This test, and its appropriateness for examining rat sera, has been described in detail elsewhere (LeDuc et al., 1984; Childs et al., 1985). Duplicate dilutions of sera were initially tested at 1:8 and 1:32; samples positive at 1:32 were titrated to endpoint.

During the MRR study, survivorship data were generated by summing monthly intervals during which rats were known to be alive. Survival, as defined by this technique, is a minimum estimate, as rats were at least 1- to 2-mo-old at first capture, many would live past their last capture, and some become trap-shy and therefore disappear from the trappable population. Rats seroconverting from negative to positive between successive recaptures were not included in the survival analysis, although their survival is described. Rats seroreverting from positive to negative were included in all analyses, as these animals were typically small, and their low antibody titers were presumed to be due to maternal antibody rather than to viral infection (Childs et al., 1987 a, b).

Growth rates were computed by obtaining

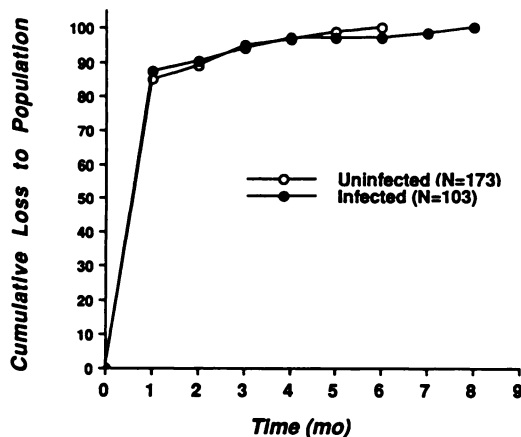


FIGURE 1. Cumulative loss of tagged seronegative and seropositive rats from the marked and released population of *Rattus norvegicus*. The time interval (mo) represents successive trapping sessions of 3 days.

differences in body weights and lengths for individual recaptured animals. Growth rates were stratified by weight at first capture but not by other attributes. Five of 57 growth measurements of body length were assigned a zero value, as these animals showed negative body length growth with time, presumably as a result of mismeasurement. These five individuals were large (>300 g) adults at the time of their second measurement, and four of these were >300 g at first capture, so their growth rates were expected to be small (Glass et al., 1988b, 1989).

Total embryo counts for seropositive and seronegative female rats were compared by analysis of covariance (ANCOVA; Snedecor and Cochran, 1967), using body weight or length as the covariate. Previous studies have demonstrated significant variation in fertility with body weight in this population of rats (Glass et al., 1989). A similar ANCOVA was performed on testes volumes for males, as this measure has also been shown to increase with size (Glass et al., 1989).

Analyses of size characteristics of infected and uninfected rats involved sex-stratified comparisons, by regression and ANCOVA, of \log_{10} body weight on length. All data analyses were performed using SAS software (Statistical Analysis System Institute Inc., 1985), and numbers in the text are reported as means \pm 1 standard error (SE), unless otherwise specified.

RESULTS

Prevalence of infections

Six hundred fifty-five individual rats (344 males, 311 females) were captured from residential locations, of which 51%

of males and 54% of females had hantaviral antibody titers $\geq 1:32$. One hundred seventy-three uninfected rats and 103 infected rats were captured on one or more occasions during the MRR study. An additional 17 rats seroconverted during successive sampling intervals and were excluded from the survival analysis. Prevalence of hantaviral antibody varied significantly among the sites (36 to 100%; Childs et al., 1987a, b), and the discrepancy between the overall seropositivity rate and the rate for the MRR study population reflected the choice of study locations not a difference in rat recapture potential associated with infection.

Survival of uninfected and infected rats

The survival of uninfected and infected rats was essentially identical (Fig. 1; data shown as cumulative loss to trappable population over time). The mean recapture interval (assigning 0's to animals never recaptured) was 0.37 ± 0.07 (SE) mo for seronegative rats ($n = 173$) and 0.39 ± 0.13 mo for seropositive rats ($n = 103$) and there was no significant difference between the two distributions (Wilcoxon test, $z = -0.32$, $P > 0.75$). Single captures accounted for 85% of all uninfected and 87% of all infected rat captures. Consideration of only multiple recaptures of animals yielded mean survival intervals of 2.4 ± 0.25 mo ($n = 26$) and 3.1 ± 0.61 mo ($n = 13$) for seronegative and positive individuals, respectively. Uninfected rats were recaptured at a maximum of 5 mo, compared to 7 mo for infected rats. Individual rats which seroconverted were followed for up to 13 mo.

Growth rates

Growth rates (body weight and length) were computed from 30 recaptures of seronegative rats and 27 recaptures of seropositive or seroconverting rats (some individuals contributed two values to this analysis). Seroconverting rats were included in this analysis because the time-lag in the development of hantaviral antibody

TABLE 1. Growth rates (g/mo, mm/mo (\pm SD)) of seropositive and seronegative *Rattus norvegicus* from residential locations in Baltimore, Maryland. Rats are stratified on the basis of body weight at first capture.

Variable	Serological status	Weight at first capture	
		≤ 200 g	> 200 g
Weight (n)	seronegative	69.7 \pm 28.5 (19)	41.3 \pm 48.2 (11)*
	seropositive	66.1 \pm 18.1 (5)	15.7 \pm 22.2 (22)
Body length (n)	seronegative	19.0 \pm 6.8 (19)	6.8 \pm 6.3 (11)
	seropositive	23.1 \pm 9.0 (5)	5.7 \pm 6.1 (22)

*Growth rate for uninfected rats > 200 g is greater than that for infected rats, $t = 2.14$, $P = 0.04$.

following infection in rats (on the order of 2 wk; Lee et al., 1986) is considerably shorter than the sampling period (1 mo). In addition, the mean interval between successive captures of seroconverting rats was 3.9 ± 0.65 mo, so including these data should allow identification of growth rate changes accompanying acute and early infection. Data were stratified into two groups on the basis of weight at first capture: ≤ 200 g, considered to be sexually immature animals (Glass et al., 1989), and > 200 g considered to be young adult to adult rats (Table 1). Growth rates for the smaller weight class were indistinguishable. Uninfected rats in the larger weight class grew in body weight more than twice as fast as their counterparts ($t = 2.14$, $df = 32$, $P = 0.04$). However, growth rates in body length were similar. Conclusive interpretation of these data is precluded because significantly more uninfected rats were sampled during cold months (November to May) than during warm months (June to October) compared to infected animals ($\chi^2 = 7.20$, $P = 0.007$). Weight gains in residential locations are greatest during the cold season, and this could have contributed to the larger value for uninfected rats (Glass et al., 1988b).

Size of uninfected and infected rats

Although the growth rate data suggest infected rats may grow more slowly in weight than uninfected rats, this difference is not reflected by body weight and length relationships of individuals. AN-

COVA comparing \log_{10} body weights between seronegative and seropositive animals, with body length as the covariate, revealed no differences (Table 2). Uninfected male rats had a mean \log_{10} body weight of 2.37 ± 0.02 g and mean body length of 213.3 ± 2.77 mm, compared to 2.47 ± 0.03 g and 226.7 ± 3.85 mm for infected males (slope, $F = 0.39$, $df = 3,250$, $P = 0.44$; intercept, $F = 0.89$, $df = 2,251$, $P = 0.35$). Uninfected female rats had a mean \log_{10} body weight of 2.33 ± 0.02 g and a mean body length of 205.9 ± 2.65 mm, compared to 2.37 ± 0.03 g and 213.3 ± 4.69 mm for infected females (slope, $F = 1.57$, $df = 3,222$, $P = 0.21$; intercept, $F = 0.57$, $df = 2,223$, $P = 0.45$).

Sexual maturity and fertility of female rats

There was no obvious difference in external evidence of reproductive maturity of seronegative versus seropositive female rats in different weight groupings (Table 3). Overall, 49% of female rats ≤ 200 g had perforate vaginal orifices compared to 98% in animals > 200 g. Fifty-four of 183 (30%) female rats necropsied were pregnant, of which 35 (65%) were antibody positive. The number of embryos carried by seropositive females ($\bar{x} = 11.0 \pm 0.6$; range 1 to 16) was slightly greater than that for negative females ($\bar{x} = 10.7 \pm 0.8$; range 6 to 16; Fig. 2). However, ANCOVA revealed no significant difference in weight-specific embryo counts (slope, $F = 0.58$, $df = 3,50$, $P = 0.58$; intercept, $F = 0.21$, $df = 2,51$, $P = 0.65$) or length specific

TABLE 2. Regression parameters of log₁₀ body weight on body length for *Hantavirus* seropositive and seronegative Norway rats (*Rattus norvegicus*) from Baltimore, Maryland.

Sex	Serological status	Slope ^a	Intercept ^a	r ^b
Male	negative	0.0069	0.90	0.94
Male	positive	0.0067	0.95	0.95
Female	negative	0.0071	0.87	0.97
Female	positive	0.0067	0.93	0.97

^a Slope and intercept between seronegative and seropositive individuals of both sexes are insignificant (ANCOVA; see text).

^b All regression lines significant at *P* < 0.0001.

embryo counts (slope, *F* = 1.04, *df* = 3, 12, *P* = 0.33; intercept, *F* = 0.22, *df* = 2, 13, *P* = 0.65).

Sexual maturity and testes size in male rats

Scrotal testes were found at similar frequency in uninfected versus infected rats of different size classes (Table 3). Overall, 52% of males ≤200 g were scrotal compared to ~100% of males >200 g. Testes volumes of 45 negative and 29 positive rats were similar (\bar{x} = 1,548 ± 95.5 mm³, and \bar{x} = 1,728.9 ± 138.1 mm³, respectively), and ANCOVA revealed no significant differences in size with either weight (slope, *F* = 0.00, *df* = 3, 70, *P* = 0.97; intercept, *F* = 0.12, *df* = 2, 71, *P* = 0.73) or body length (slope, *F* = 1.20, *df* = 3, 70, *P* = 0.28; intercept, *F* = 0.00, *df* = 2, 71, *P* = 0.98) as covariates.

TABLE 3. External evidence of sexual maturation in seropositive and seronegative rats (*Rattus norvegicus*) tested for antibody to a *Hantavirus*.

	Weight class	
	≤200 g	>200 g
% Perforate females (<i>n</i>)		
Seronegative	52 (71)	98 (90)
Seropositive	43 (37)	99 (92)
% Scrotal males (<i>n</i>)		
Seronegative	50 (54)	100 (106)
Seropositive	57 (30)	99 (116)

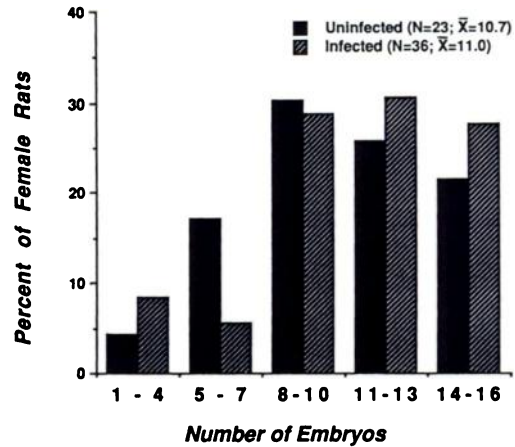


FIGURE 2. Frequency (percent) histogram of litter sizes for seronegative and seropositive *Rattus norvegicus*, collected from Baltimore, Maryland, from 1980 to 1988.

DISCUSSION

The field data described herein suggest that hantaviral infection does not influence demographic or physiologic processes, as defined by individual survival, growth or fertility, in natural populations of Norway rats, and support similar findings obtained from laboratory infections in natural host species (Lee et al., 1981; Yanagihara et al., 1985). However, several caveats must be mentioned before proceeding to a specific discussion. First, these data were obtained by live trapping rats, so that suckling and juvenile animals (<40 g) were poorly represented. Infection, possibly resulting in mortality, in this youngest segment of the population was not addressed. Second, survival estimates were based on recapture of a small fraction (14% of released animals) of the population over a portion of their lifespans. Third, embryo counts were based on all stages of pregnancy; if fetal wastage occurred only in near term pregnancies then effects of infection may be overlooked. Finally, some pooling of data across seasons and size classifications was made to permit statistical testing for trends where sample sizes would not permit more rigorous analysis.

In certain experimental situations, hantaviruses inoculated intracranially, intraperitoneally, or subcutaneously into suckling mice (*Mus musculus*; Tsai et al., 1982; Kim and McKee, 1985; McKee et al., 1985) or suckling rats (*R. norvegicus*; Tanishita et al., 1986; Zhang et al., 1988) can cause fatal disease, although the significance of these observations in natural situations is unclear. Field data suggest transmission of hantaviruses occurs primarily in young adult to adult animals (Arikawa et al., 1986; Verhagen et al., 1986; Childs et al., 1987a), a life stage when significant pathology may not result from infection. One mechanism to explain this natural pattern in transmission is the finding that passively acquired maternal antibody protects young rats from hantaviral challenge for up to 10 wk postbirth, even when antibody is no longer detectable by serological exam (Zhang et al., 1988). Breeding in rats is size and age dependent, and a large fraction of breeding females is already seropositive (up to 90%, Childs et al., 1987b; Glass et al., 1989). Therefore, a majority of newly born rats may be protected during potentially vulnerable periods of early life. In addition, temporary, maternally derived herd immunity may reduce and postpone transmission among cohorts of newly weaned animals.

Survival of infected rats appears no different from that of uninfected rats, and these animals reached sexual maturity, bred and conceived in a normal fashion (Table 3 and Figs. 1, 2). The percentage of seropositive animals captured once (87%) was not different from that found for uninfected animals (85%), suggesting that infection did not influence survival or trapability of this species. In the only other longitudinal study of a hantaviral infection in rodents, Verhagen et al. (1986) concluded that *C. glareolus* survival was uninfluenced by infection with Puumala virus, a related *Hantavirus*. Hantaviral infection thus appears to have no impact on the survival of infected, postweaning

rodents in the two host-virus systems studied in detail.

A more subtle effect on lifetime reproductive output could result from retardation in growth, since reproductive maturity in rats and other rodents may be more closely correlated with body size than with chronological age (Bronson, 1987; Glass et al., 1989). Recent studies on small mammals have indicated the importance of body weight as a demographic variable (Sauer and Slade, 1985, 1986). Our data suggest that growth, as measured by body weight but not length, may be retarded in infected rats >200 g (Table 1). However, this difference was not ultimately reflected in the overall size relationships of infected compared to uninfected animals in the population (Table 2). The numbers used to compare growth rates in our study were small, and the analyses did not attempt to distinguish between sexual or seasonal factors that play a role in growth rates (Glass et al., 1989). However, since the relationships between body weight and body length are similar for infected and uninfected rats, the quality of growth or condition of these animals was likely comparable.

Seropositive and seronegative females had similar embryo counts, suggesting that conception and fertility were not influenced by infection. These data would not reflect differences in the survival of embryos to full term, or in perinatal survival of young rats from infected versus uninfected females.

Chronic infection and malnutrition are factors that can influence testes size and, presumably, spermatogenesis in humans (Handelsman and Staraj, 1985). Testes weight, or volume, is also an excellent indicator of spermatid production in rodents (Chubb and Nolan, 1985). Our examinations of testes sizes did not indicate any adverse effects of hantaviral infection on testes volume, and, presumably, reproductive capabilities of infected males were normal.

The conclusion of these analyses is that

hantaviruses do not appear to influence the survival, size structure or fertility of Norway rats. These findings are of particular interest when considering the mechanisms by which viruses are maintained in populations, as they represent a marked contrast to other patterns of viral persistence as exemplified by the South American arenaviruses, Machupo and Junin. In this system, immunotolerant *Calomys* spp. that are persistently viremic and viruric may be at a competitive disadvantage because of fatal in utero transmission to embryos, decreased survival of live-born offspring, and growth retardation in juveniles (Webb et al., 1975; Vitullo et al., 1987). As immunotolerance or immunocompetence may be genetically controlled, Machupo and Junin viruses can ultimately influence the fitness of individual rodents and the genetic structure of exposed populations (Johnson, 1985).

The data reported here primarily reflect the impact of hantaviral infections acquired by juvenile or adult Norway rats. Additional experimental information on the effects of challenge to young rats, born to females exposed to a *Hantavirus* during different intervals prior to breeding, is clearly needed. However, information to date suggests that hantaviruses play an insignificant role in the demographic processes of rat populations.

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In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to

reflect the position of the U.S. Department of the Army of the U.S. Department of Defense.

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