Individuals often differ in their ability to transmit disease and identifying key individuals for transmission is a major issue in epidemiology. Male hosts are often thought to be more important than females for parasite transmission and persistence. However, the role of infectious females, particularly the transient immunity provided to offspring through maternal antibodies (MatAbs), has been neglected in discussions about sex-biased infection transmission. We examined the effect of host sex upon infection dynamics of zoonotic Puumala hantavirus (PUUV) in semi-natural, experimental populations of bank vole (Myodes glareolus). Populations were founded with either females or males that were infected with PUUV, whereas the other sex was immunized against PUUV infection. The likelihood of the next generation being infected was lower when the infected founders were females, underlying the putative importance of adult males in PUUV transmission and persistence in host populations. However, we show that this effect probably results from transient immunity that infected females provide to their offspring, rather than any sex-biased transmission efficiency per se. Our study proposes a potential contrasting nature of female and male hosts in the transmission dynamics of hantaviruses.

1. Introduction

Often a minority of infected hosts is responsible for the majority of the transmission and persistence of pathogens in host populations [1–3]. Recently, host sex has gained attention as a key characteristic that determines an individual’s role in disease transmission, with male hosts apparently more important than females [2,3]. Typical explanations for this sex-biased effect lie in male characteristics during breeding season; for example, males may occupy larger home ranges, encounter more aggressive contacts and excrete infectious particles more or longer than females, increasing their likelihood of encountering infections as well as spreading them further [4–6]. However, another important determinant of disease transmission is likely to be maternal transfer of transient immunity to offspring [7–9]. Maternal antibody (MatAb) protection affects, for example, Puumala hantavirus (PUUV) infection dynamics in natural populations [10,11], but the contribution of MatAbs to sex-biased transmission of disease is not known.
Hantaviruses are found in several mammalian orders, but only rodent-borne hantaviruses cause diseases in humans [12]. PUUV causes a mild haemorrhagic fever with renal syndrome, with thousands of human cases diagnosed annually in Europe [13]. The host of PUUV is the bank vole (Myodes glareolus) [13]. PUUV infection in bank vole is asymptomatic and chronic [14]. Infected bank voles mount an immune response with lifelong antibody production [15]. The transmission of PUUV is horizontal [15]. In the breeding season, infection is more common in adult male bank voles than adult females [11,16], implying that they are more exposed and/or more susceptible to infection than the females. Infected female bank voles transfer MatAbs to their progeny, with MatAbs remaining at detectable levels up to the age of eight weeks [10,15]. In September, however, a young individual (approx. 1.5 months) may have been seropositive due to infection or due to MatAbs. Therefore, a seropositive individual was carrying MatAbs in September if it subsequently was seronegative in November. However, an individual that was seropositive both in September and in November could have been a MatAb carrier or infected in September; these animals (n = 14) were excluded when the probability of carrying MatAbs and MatAb prevalence in September were estimated (see electronic supplementary material, tables S1 and S2).

Generalized linear mixed models (GLMM) with binomial distributions and logit link functions (‘lmer’ function in ‘lme4’ package in R software (http://www.r-project.org/)) were used to assess the effect of treatment (FI and MI) and sex on the probability of an enclosure-born individual being PUUV seropositive (September), carrying MatAbs (September; reduced data) and being PUUV infected (November). Moreover, PUUV infection likelihood in November was examined in relation to the preceding MatAb prevalence (based on reduced data). Enclosure was a random factor in all analyses.

### 2. Material and methods

Laboratory-born offspring of wild-captured bank voles (Central Finland 62°37'N, 26°20'E) were used as founder individuals, which were either immunized against PUUV infection (with baculovirus-expressed recombinant PUUV nucleocapsid protein [17]) or mock-immunized. Later, the mock-immunized individuals were experimentally infected with PUUV, whereas immunized animals were mock-infected (details in the electronic supplementary material). Two founder females and two males were released in each of the 10 outdoor enclosures (each 0.2 ha) in mid-July 2004. In five enclosures, the females were infected and the males were immunized (FI treatment). In the other five enclosures, the males were infected and the females were immunized (MI treatment). PUUV infection was followed using serological methods among the progeny of the founders, with sampling approximately 1.5 months (September) and approximately three months (November) after birth. At the age of approximately three months, a seropositive result was interpreted as truly infected as the animals at this age are too old to carry MatAbs [10,15]. In September, however, a young individual (approx. 1.5 months) may have been seropositive due to infection or due to MatAbs. Therefore, a seropositive individual was carrying MatAbs in September if it subsequently was seronegative in November. However, an individual that was seropositive both in September and in November could have been a MatAb carrier or infected in September; these animals (n = 14) were excluded when the probability of carrying MatAbs and MatAb prevalence in September were estimated (see electronic supplementary material, tables S1 and S2).

#### Table 1. Effect of treatment (MI, founder males infected) and sex on the likelihood of enclosure-born individuals being (a) PUUV seropositive (n = 77) and (b) MatAb carrier (n = 63) in September and (c) PUUV infected in November (n = 77). Intercept represents an enclosure-born female in FI (i.e. founder females infected) treatment. Parameter estimates (logit scale) are based on GLMMs. Variance of the enclosure = \( \sigma^2 \), s.d. = standard deviation of \( \sigma^2 \).

<table>
<thead>
<tr>
<th>covariate</th>
<th>coefficient (s.e.)</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) PUUV seropositive in September</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intercept</td>
<td>1.891(0.818)</td>
<td>2.313</td>
<td>0.021</td>
</tr>
<tr>
<td>treatment (MI)</td>
<td>-2.793(0.985)</td>
<td>-2.836</td>
<td>0.005</td>
</tr>
<tr>
<td>sex (male)</td>
<td>0.564(0.667)</td>
<td>0.846</td>
<td>0.398</td>
</tr>
<tr>
<td>random effect: enclosure</td>
<td>( \sigma^2 = 1.212; )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) MatAb positive in September</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intercept</td>
<td>1.761(0.794)</td>
<td>2.218</td>
<td>0.027</td>
</tr>
<tr>
<td>treatment (MI)</td>
<td>-3.629(0.982)</td>
<td>-3.697</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sex (male)</td>
<td>0.481(0.797)</td>
<td>0.604</td>
<td>0.546</td>
</tr>
<tr>
<td>random effect: enclosure</td>
<td>( \sigma^2 = 0.933; )</td>
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<tr>
<td>(c) PUUV infection in November</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intercept</td>
<td>-1.558(0.654)</td>
<td>-2.381</td>
<td>0.017</td>
</tr>
<tr>
<td>treatment (MI)</td>
<td>2.058(0.755)</td>
<td>2.724</td>
<td>0.007</td>
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<tr>
<td>sex (male)</td>
<td>0.246(0.583)</td>
<td>0.422</td>
<td>0.673</td>
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<tr>
<td>random effect: enclosure</td>
<td>( \sigma^2 = 0.607; )</td>
<td></td>
<td>0.779</td>
</tr>
</tbody>
</table>

3. Results

Forty-nine out of 77 (64%) enclosure-born bank voles were PUUV seropositive when they were approximately 1.5 months old (in September), and the likelihood of being PUUV seropositive was significantly (\( p = 0.005 \)) higher when the founder females were infected (FI treatment) than when founder males were infected (MI treatment; table 1a).
Most seropositives (35/49 = 71%) carried MatAbs (i.e. turned seronegative in November) and the likelihood of carrying MatAbs was significantly higher in the FI treatment ($p < 0.001$, table 1b and figure 1a).

At approximately three months, 44% (34/77) of enclosure-born individuals were infected, with a significantly ($p = 0.007$) increased likelihood of the young being infected in the MI treatment in comparison with the FI treatment (table 1c and figure 1b).

MatAbs clearly impacted PUUV transmission as infection risk for individuals approximately three months old was negatively related to MatAb prevalence in the population in September (GLMM, coefficient estimate (logit scale) = $-0.032$, s.e. $\pm 0.011$, $z = -2.818$, $p = 0.005$; figure 2).

4. Discussion

Here, we provide experimental evidence that adult male bank voles are more effective transmitters of PUUV to young individuals than adult females: in November, PUUV infection was more common in the next generation when the infectious founders were males (MI treatment) than when they were females (FI treatment). However, this sex-biased transmission does not appear to be entirely due to a superior male transmission capacity per se, because the likelihood of the young carrying MatAbs was high in the FI treatment, and the probability of PUUV infection in November was negatively related to the preceding (September) MatAb prevalence. Thus, our results suggest that infected breeding females impact PUUV dynamics by delaying its transmission in the host population through the protection given to their offspring.

In natural host populations, high PUUV infection prevalence in breeding females results in a high MatAb prevalence in young individuals, which is followed by delayed and low infection prevalence [10]. High MatAb prevalence may increase the risk of PUUV of disappearing from the host population, owing to a shortage of susceptible individuals. Our finding supports this idea as in one of the FI treatment replicates, MatAb prevalence was 100% in September and no PUUV-infected young (out of seven young individuals) were found in November. Consequently, long-term persistence of PUUV is likely to depend on the presence of chronically infectious older individuals until MatAb-protected individuals become first susceptible and then infected.

It is not entirely clear whether the chronically infected old individuals, likely to be the key individuals for the long-term persistence of PUUV, are males. On the one hand, old male bank voles might transmit PUUV more than females, as they have higher infection prevalence [10,16] and larger overlapping home ranges [18]. They may also encounter more aggressive contacts and shed virus longer, as has been seen in overlapping home ranges [18]. They may also encounter more aggressive contacts and shed virus longer, as has been seen in other hantavirus–host systems [5,6]. On the other hand, the lower survival rate of males compared with females [19] may reduce their contribution to the long-term persistence of PUUV. Moreover, a study on key host individuals in another hantavirus–rodent host system (Sin Nombre hantavirus—Peromyscus maniculatus) revealed that virus transmission was driven by a minority of heavy (i.e. old) individuals, largely regardless of sex [20], further questioning the importance of males per se.

To conclude, adult female and male bank vole hosts have contrasting roles in the transmission of PUUV to the next generation: infected females provide initial protection that delays the influx of susceptible individuals, and consequently reduces infection, while males might have important roles in overcoming the effect of maternal antibodies and maintaining PUUV transmission and persistence in host populations. This study highlights the complexity behind individual variation...
in efficacy of disease transmission and the need to further examine the interaction between host sex, age and breeding status upon the role of host sex in driving the dynamics of infections in host populations.

This research adhered to the Association for the Study of Animal Behaviour/Animal Behavior Society Guidelines for the Use of Animals in Research, the legal requirements in Finland, and institutional guidelines.

References