biology letters

rsbl.royalsocietypublishing.org

Research

Cite this article: Kallio ER, Henttonen H, Koskela E, Lundkvist Å, Mappes T, Vapalahti O. 2013 Maternal antibodies contribute to sex-based difference in hantavirus transmission dynamics. Biol Lett 9: 20130887. http://dx.doi.org/10.1098/rsbl.2013.0887

Received: 15 October 2013
Accepted: 29 November 2013

Subject Areas:
ecology

Keywords:
host sex, Puumala hantavirus, bank vole, maternal antibody, transmission

Author for correspondence:
Eva R. Kallio
e-mail: eva.kallio@jyu.fi

†Authors after the first are in alphabetical order.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2013.0887 or via http://rsbl.royalsocietypublishing.org.

Royal Society Publishing
Informing the science of the future

Pathogen biology

Maternal antibodies contribute to sex-based difference in hantavirus transmission dynamics

Eva R. Kallio[1,2,†], Heikki Henttonen[3], Esa Koskela[1], Åke Lundkvist[4,5], Tapio Mappes[1] and Olli Vapalahti[6,7,8]

[1]Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, 40014 University of Jyväskylä, Finland
[2]Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK
[3]Vantaa Research Unit, Finnish Forest Research Institute, PO Box 18, 01301 Vantaa, Finland
[4]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden
[5]Swedish Institute for Communicable Disease Control and Karolinska Institutet, 17182 Solna, Sweden
[6]Department of Virology, Haartman Institute, PO Box 21, 00014 University of Helsinki, Finland
[7]Department of Veterinary Sciences, PO Box 66, University of Helsinki, 00014 Finland
[8]HUSLAB, PO Box 400, 00029 HUS, Helsinki, Finland

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.

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 σ^2.

covariate		coefficient (s.e.)	*z*-value	*p*-value
(*a*) PUUV seropositive in September				
intercept		1.891(0.818)	2.313	0.021
treatment	(MI)	−2.793(0.985)	−2.836	0.005
sex	(male)	0.564(0.667)	0.846	0.398
random effect: enclosure		$\sigma^2 = 1.212$; s.d. = 1.101		
(*b*) MatAb positive in September				
intercept		1.761(0.794)	2.218	0.027
treatment	(MI)	−3.629(0.982)	−3.697	<0.001
sex	(male)	0.481(0.797)	0.604	0.546
random effect: enclosure		$\sigma^2 = 0.933$; s.d. = 0.966		
(*c*) PUUV infection in November				
intercept		−1.558(0.654)	−2.381	0.017
treatment	(MI)	2.058(0.755)	2.724	0.007
sex	(male)	0.246(0.583)	0.422	0.673
random effect: enclosure		$\sigma^2 = 0.607$; s.d. = 0.779		

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].

We studied whether female and male bank voles have different roles in PUUV transmission, particularly through the effect of MatAbs. We hypothesize that the protection provided to young by infected females reduces or delays PUUV transmission and decreases this pathogen's persistence in host populations. We used a reciprocal experimental design where founder individuals of one sex were immunized and the other sex were infected prior to the release into outdoor enclosures (five replicates per treatment) during the breeding season. PUUV infection was followed among the progeny until they were approximately three months old.

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 an 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.

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 1*a*).

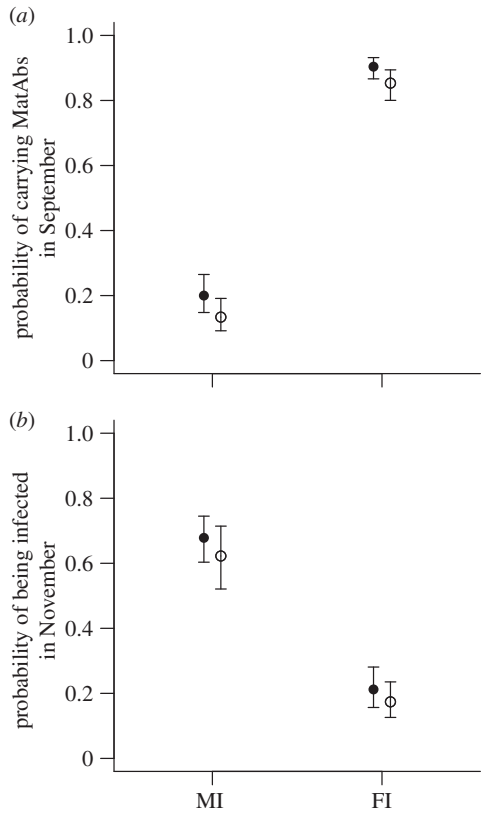

Figure 1. Predicted probability of enclosure-born individuals (*a*) carrying MatAbs at the age of approximately 1.5 months and (*b*) being PUUV infected at the age of approximately three months in relation to treatment (MI, founder males infected; FI, founder females infected) and individual sex (females, open dots; males, filled dots). Error bars represent 95% CI, averaged over random effect.

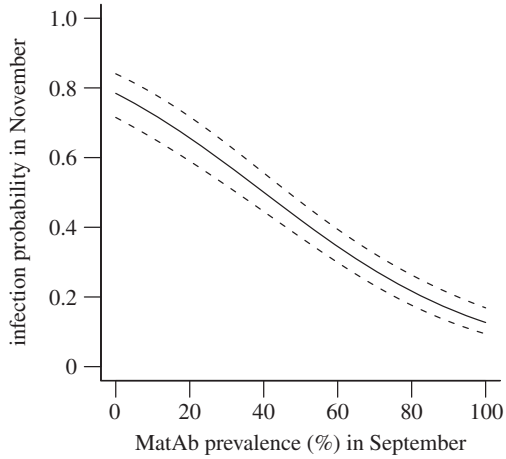

Figure 2. Predicted probability (solid line) of enclosure-born individuals being PUUV infected at the age of approximately three months (November) in relation to MatAb prevalence in the population in September (dashed lines represent 95% CI, averaged over random effect). Predictions are based on GLMM (details in the text).

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 1*b* and figure 1*a*).

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 1*c* and figure 1*b*).

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. ± 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 and 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.

Acknowledgements. We thank Antti Poikonen, Leena Kostamovaara, Steve Paterson, Mike Begon, Greg Hurst and Phill Watts for help and valuable comments; Konnevesi Research Station for fieldwork facilities.

Data accessibility. Data are available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.d632k [21].

Funding statement. This study was financially supported by the Academy of Finland (grant nos. 63789, 202166, 206091 to T.M., 257340 to E.K., 250524 to E.R.K.), and partially supported by EU grant FP7-261504 EDENext and catalogued as EDENext172 (http://www.edenext.eu).

References

1. Woolhouse ME *et al*. 1997 Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proc. Natl Acad. Sci. USA* **94**, 338–342. (doi:10.1073/pnas.94.1.338)
2. Perkins SE, Cattadori IM, Tagliapietra V, Rizzoli AP, Hudson PJ. 2003 Empirical evidence for key hosts in persistence of a tick-borne disease. *Int. J. Parasitol.* **33**, 909–917. (doi:10.1016/S0020-7519(03)00128)
3. Ferrari N, Cattadori I, Nespereira J, Rizzoli A, Hudson P. 2004 The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. *Ecol. Lett.* **7**, 88–94. (doi:10.1046/j.1461-0248.2003.00552.x)
4. Skorping A, Jensen KH. 2004 Disease dynamics: all caused by males? *Trends Ecol. Evol.* **19**, 219–220. (doi:10.1016/j.tree.2004.02.006)
5. Klein SL, Calisher CH. 2007 Emergence and persistence of hantaviruses. In *Wildlife and emerging zoonotic diseases: the biology, circumstances and consequences of cross-species transmission* (eds JE Childs *et al*.), pp. 217–252. Berlin, Germany: Springer.
6. Mills JN, Ammam BR, Glass GE. 2010 Ecology of hantaviruses and their hosts in North America. *Vector Borne Zoonot.* **10**, 563–574. (doi:10.1089/vbz.2009.0018)
7. Grindstaff JL, Brodie ED, Ketterson ED. 2003 Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proc. R. Soc. Lond. B* **270**, 2309–2319. (doi:10.1098/rspb.2003.2485)
8. Boulinier T, Staszewski V. 2008 Maternal transfer of antibodies: raising immuno-ecology issues. *Trends Ecol. Evol.* **23**, 282–288. (doi:10.1016/j.tree.2007.12.006)
9. Hasselquist D, Nilsson JA. 2009 Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. *Phil. Trans. R. Soc. B* **364**, 51–60. (doi:10.1098/rstb.2008.0137)
10. Kallio ER, Poikonen A, Vaheri A, Vapalahti O, Henttonen H, Koskela E, Mappes T. 2006 Maternal antibodies postpone hantavirus infection and enhance individual breeding success. *Proc. R. Soc. B* **273**, 2771–2776. (doi:10.1098/rspb.2006.3645)
11. Kallio ER, Begon M, Henttonen H, Koskela E, Mappes T, Vaheri A, Vapalahti O. 2010 Hantavirus infections in fluctuating host populations: the role of maternal antibodies. *Proc. R. Soc. B* **277**, 3783–3791. (doi:10.1098/rspb.2010.1022)
12. Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S, Mustonen J. 2013 Uncovering the mysteries of hantavirus infections. *Nat. Rev. Micro.* **11**, 539–550. (doi:10.1038/nrmicro3066)
13. Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, Vaheri A. 2003 Hantavirus infections in Europe. *Lancet Infect. Dis.* **3**, 653–661. (doi:10.1016/S1473-3099(03)00774-6)
14. Meyer BJ, Schmaljohn CS. 2000 Persistent hantavirus infections: characteristics and mechanisms. *Trends Microbiol.* **8**, 61–67. (doi:10.1016/S0966-842X(99)01658-3)
15. Gavrilovskaya IN, Apekina NS, Bernshtein AD, Demina VT, Okulova NM, Myasnikov YA, Chumakov MP. 1990 Pathogenesis of hemorrhagic fever with renal syndrome virus infection and mode of horizontal transmission of hantavirus in bank voles. *Arch. Virol.* **110**(Suppl. 1), 57–62.
16. Olsson GE, White N, Ahlm C, Elgh F, Verlemyr AC, Juto P, Palo RT. 2002 Demographic factors associated with hantavirus infection in bank voles (*Clethrionomys glareolus*). *Emerg. Infect. Dis.* **8**, 924–929. (doi:10.3201/eid0809.020037)
17. Lundkvist A, Kallio-Kokko H, Sjolander KB, Lankinen H, Niklasson B, Vaheri A, Vapalahti O. 1996 Characterization of Puumala virus nucleocapsid protein: identification of B-cell epitopes and domains involved in protective immunity. *Virology* **216**, 397–406. (doi:10.1006/viro.1996.0075)
18. Bondrup-Nielsen S, Karlsson F. 1985 Movements and spatial patterns in populations of *Clethrionomys* species: a review. *Ann. Zool. Fenn.* **22**, 385–392.
19. Kallio ER, Voutilainen L, Vapalahti O, Vaheri A, Henttonen H, Koskela E, Mappes T. 2007 Endemic hantavirus infection impairs the winter survival of its rodent host. *Ecology* **88**, 1911–1916. (doi:10.1890/06-1620.1)
20. Clay CA, Lehmer EM, Previtali A, St Jeor S, Dearing MD. 2009 Contact heterogeneity in deer mice: implications for Sin Nombre virus transmission. *Proc. R. Soc. B* **276**, 1305–1312. (doi:10.1098/rspb.2008.1693)
21. Kallio ER, Henttonen H, Koskela E, Lundkvist A, Mappes T, Vapalahti O. 2013 Data from: maternal antibodies contribute to sex based difference in hantavirus transmission dynamics. Dryad Digital Repository: http://doi.org/10.5061/dryad.d632k.