

Age-related effects of chronic hantavirus infection on female host fecundity

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Summary

1. Pathogens often cause detrimental effects to their hosts and, consequently, may influence host population dynamics that may, in turn, feed back to pathogen transmission dynamics. Understanding fitness effects of pathogens upon animal host populations can help to predict the risks that zoonotic pathogens pose to humans.

2. Here we determine whether chronic infection by Puumala hantavirus (PUUV) affects important fitness-related traits, namely the probability of breeding, reproductive effort and mother and offspring condition, in the bank vole (*Myodes glareolus*). Using 9 years empirical data in a PUUV endemic area in Central Finland, we found differences between reproductive characteristics of PUUV-infected and uninfected female bank voles.

3. Young infected females had a significantly higher, and old individuals lower, likelihood of reproducing than uninfected animals during the middle of the breeding season. The implication is that PUUV infection may have long-term deleterious effects that are observed at old age, while in young individuals, the infection may enhance breeding probability by directing resources towards current breeding.

4. Moreover, PUUV infection was related with the mother's body condition. Infected mothers were in poorer condition than uninfected mothers in the early breeding season, but were in better condition than uninfected mothers during the middle of the breeding season. Offspring body condition was positively associated with mother's body condition, which, in turn, was related to the PUUV infection status of the mother.

5. Our findings indicate that chronic infection may affect the reproduction of female hosts, but the effect is dependent on the host age. The effect of chronic hantavirus infection was small and density-independent and hence unlikely to contribute to the cyclic population dynamics of the host. However, the effects on a female's reproductive output might affect the abundance of young susceptible individuals in the population and hence influence the transmission and persistence of the pathogen. Although experimental and long-term capture–mark–recapture studies are required to further clarify the fitness effects of hantavirus infection and their consequences for pathogen dynamics, this study shows that the infection may have complex effects that are dependent on the age of the individual and the time of the breeding season.

Key-words: disease transmission, endemic pathogen, female fecundity, host–pathogen interaction, resource allocation

Introduction

Endemic pathogens, which do not cause clinical illness in their hosts, may still affect fitness by reducing the survival

and/or reproductive rates of infected individuals (e.g. Feore *et al.* 1997; Telfer *et al.* 2002, 2005; Burthe *et al.* 2006, 2008; Kallio *et al.* 2007). Consequently, endemic pathogens may alter the demography and dynamics of their host populations, with any such impact potentially feeding back to affect the transmission dynamics of the pathogen itself (e.g. Anderson & May 1978; May & Anderson 1978; Tompkins & Begon 1999; Begon *et al.*

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2008; Smith *et al.* 2008). Understanding these interactions has fundamental epidemiological relevance when the pathogen is zoonotic, as the disease dynamics in the host population determine the risks posed to humans (e.g. Mills & Childs 1998; Matthews & Woolhouse 2005).

Rodents are the host species to many wildlife-borne zoonotic pathogens (Mills & Childs 1998; Ostfeld & Holt 2004; Woolhouse & Gowtage-Sequaria 2005). Often rodent populations show a combination of seasonal and multiannual fluctuations in abundance (Stenseth 1999; Lambin, Bretagnolle & Yoccoz 2006; Korpela *et al.* 2013), which have been linked to fluctuations in the incidence of human infections by rodent-borne zoonotic diseases (reviewed by Davis, Calvet & Leirs 2005); for instance, in Northern Fennoscandia the human Puumala hantavirus (PUUV) infection epidemiology reflects the multiannual density fluctuations of the rodent host, the bank vole (*Myodes glareolus*) (Olsson *et al.* 2003; Kallio *et al.* 2009). While multiannual cyclic density fluctuations that prevail rodent populations in Northern Fennoscandia are thought to be determined predominantly by specialist mammalian predators and the voles' winter food resources (Hansson & Henttonen 1988; Huitu *et al.* 2007; Korpela *et al.* 2014), there is increasing evidence to suggest that pathogens may play an important role in the cycles (Soveri *et al.* 2000; Forbes *et al.* 2014).

Our aim was to clarify whether Puumala hantavirus (PUUV; genus *Hantavirus*, family *Bunyaviridae*) affects reproductive characteristics in female bank voles. Hantaviruses are widely distributed around the world and ~20 hantavirus species are known to cause illnesses in humans (Jonsson, Figueiredo & Vapalahti 2010). PUUV causes a mild haemorrhagic fever with renal syndrome in humans, with thousands of cases diagnosed annually in Europe (Vapalahti *et al.* 2003). While PUUV infection was long thought to be benign to the bank vole host, as no instances of clinical illness, reduced fecundity or increased mortality were observed in early laboratory or field studies (e.g. Verhagen *et al.* 1986; Gavrilovskaya *et al.* 1990; Bernshtein *et al.* 1999), recent long-term field studies have revealed that hantaviruses decrease the survival of infected rodent hosts (e.g. Kallio *et al.* 2007; Luis *et al.* 2012; Tersago *et al.* 2012). Despite this, the role of hantavirus infection in the reproductive potential of their host species has been somewhat neglected, despite numerous large-scale ecological studies on hantavirus–host systems in the wild (reviewed by e.g. Mills, Amman & Glass 2010; Olsson, Leirs & Henttonen 2010) and increasing evidence that infections mediate adverse effects on host reproduction in other pathogen–rodent host systems (Feore *et al.* 1997; Telfer *et al.* 2005; Hakkarainen *et al.* 2007). Indeed, evidence for fecundity impacts of hantaviruses is equivocal; one recent study has discussed that hantavirus infection may impair the reproductive output of female hosts (Dearing *et al.* 2009), while subsequent work suggested that PUUV

infection might increase bank voles' investment in reproduction as PUUV-infected individuals were more likely to mature than PUUV-uninfected individuals (Tersago *et al.* 2012).

We hypothesize that PUUV impairs the reproductive output of female bank vole. We used a long-term (9 year) detailed empirical data set to analyse the effect of PUUV infection on (i) probability of breeding of all females and (ii) the reproductive effort and (iii) body condition of reproduced females and (iv) offspring body condition at birth.

Materials and methods

STUDY SPECIES

In the bank vole, PUUV infection is chronic (Meyer & Schmaljohn 2000; Voutilainen *et al.* 2015) and transmitted horizontally via direct contact (Niklasson *et al.* 1995) or through contaminated environment (Kallio *et al.* 2006a). PUUV-infected females transfer maternal antibodies to their offspring, which are transiently protected against the infection (Kallio *et al.* 2006b, 2013). An infected individual creates detectable levels of antibodies 2–4 weeks after the infection, and the antibody response is persistent (Gavrilovskaya *et al.* 1990).

The bank vole is common throughout much of Europe (Amori *et al.* 2008). In Finland, the breeding season lasts from May until August–September, during which females give birth to as many as four litters that each comprises two to eight pups (Koivula *et al.* 2003). While males have large overlapping home ranges, mature females are territorial during the breeding season and their reproductive success is inversely density-dependent (Koskela, Mappes & Ylönen 1997, 1999). Bank voles are able to mature soon after weaning (3–4 weeks old), but typically only the individuals that are born early in the breeding season mature during the summer of birth whereas others delay breeding to the next year (Prévot-Julliard *et al.* 1999).

STUDY ANIMALS

Bank vole trapping sessions were carried out three times per each breeding season (BS) between 2002 and 2010 in Central Finland (62°8379 N, 26°8209 E): trappings were completed during the 'early', 'mid'- and 'late' BS during late May, in late June/early July and in late August, respectively. At each of the 20 trapping sites (distance 300–1000 m), four Ugglan Special mouse traps (Grahnb, Hillerstorp, Sweden) were situated at the corners of a 15-m square and they were set over two nights (see Kallio *et al.* 2009 for details). All captured bank voles were taken to the laboratory, without being released back to the wild. As the number of gravid females captured per trapping site per trapping session is low (zero to four individuals), the trapping protocol is not likely to affect the population dynamics in the area. Vole population abundances fluctuate cyclically in the study area, and our study period covered three full three-year-cycles ('peak' years = 2002, 2005 and 2008, 'crash/low' years = 2003, 2006 and 2009 and 'increase' years = 2004, 2007 and 2010) (Kallio *et al.* 2009, 2010). In the mid-BS trapping of 2006, serological samples were not available for females, and hence, data from this trapping period are not included in this study.

In the laboratory the sex, breeding status, weight and head width of captured individuals were recorded. We used head width as a proxy for age (Kallio *et al.* 2014), as body mass, another indicator of age in wild-captured voles (e.g. Telfer *et al.* 2008), is influenced by reproductive state in female voles. Blood samples were taken, as described by (Kallio *et al.* 2009), to allow PUUV-specific antibody detection using immunofluorescence assay (IFA) (Vapalahti *et al.* 1995). Females were held individually in standard mouse cages (43 × 26 × 15 cm, with wood shavings and hay were provided as bedding) in the laboratory (16 h L: 8 h D photoperiod at 20 ± 2 °C, food (Laboratory for 36, Lactamin AB, Stockholm, Sweden), and water were provided ad libitum) until they either gave birth or were confirmed not gravid (maximum duration of gestation is 24 days). For any females that gave birth, the number of offspring (litter size) and the body weight and head width of the pups and the mothers' body mass (postpartum weight) and head width were measured within 24 h of parturition. The time period mothers spent in the laboratory before giving birth varied between zero and 19 days (mean 10.2 days). The days in laboratory or PUUV infection status did not affect the litter size, mother's postpartum head width or body mass or average offspring head width or body mass at birth (generalized linear model results for the days in laboratory, PUUV infection status and their interaction term for all measured characteristics were not statistically significant with *P*-values >0.09).

Young bank voles may carry maternally delivered antibodies (MatAbs) against PUUV (Kallio *et al.* 2006b). We distinguished PUUV antibody positive individuals that were infected from those that were not infected (i.e. carried MatAbs) using cut-off values in body mass beyond which MatAbs do not persist (Kallio *et al.* 2010): all PUUV seropositive individuals were categorized as infected in the early BS, as they had over-wintered and were too old (i.e. heavy) to carry MatAbs; at other sampling periods only seropositive individuals with a body mass of >16.4 g (mid-BS) or >15.9 g (late BS) were considered to be PUUV infected, while the seropositive but lighter individuals and seronegative animals were regarded as uninfected (Kallio *et al.* 2010). Note that this categorization applies only to the analysis of the probability of breeding. For analyses of other reproductive characteristics, all seropositive individuals were heavier than the cut-off values for MatAbs and thus taken as PUUV-infected – the antibody status of the bred females therefore represented their infection status.

STATISTICAL ANALYSES

The focus of this study is to quantify the effect of PUUV infection on the reproductive output of female bank voles. Specifically, we characterized the effect of PUUV infection on (i) the probability of reproduction, (ii) the reproductive effort of reproducing females, (iii) the body condition of bred females and (iv) the body condition of offspring. Therefore, in all statistical analyses the PUUV infection status of the female/mother is the explanatory variable, while other predictors, which depend on the question (see details below), are included to control for possible spurious effects that leaving them out from the models could cause. A random effect *trapping session*trapping site* was included in all models to control for the potential pseudoreplication, which may result from correlation between individuals that were captured from the same site at the same time (Paterson & Lello 2003).

PROBABILITY OF BREEDING

To study the probability of a female reproducing (binary outcome), we first analysed using the entire data set whether there was any seasonal (early, mid- and late breeding season) and multiannual (low, increase and peak phase of the cycle) pattern in the dependent variable with GLMM (glmer function in lme4 package in R software, available under GNU licence at www.r-project.org) with binomial error distributions and logit-link function. Because the period of the breeding season and the phase of the cycle (and their interaction) had statistically significant effects on the reproduction probability (see Tables S1 and S2, Fig. S1, Supporting information), we further examined the effect of PUUV on the breeding probability separately for the three seasons. For each season, the probability of breeding was analysed in a similar way. First, we fitted a full model using a GLMM that included a population level predictor the phase of vole cycle, and the individual level predictors head width (mm), centred value over the females captured during each season) and PUUV infection status and all relevant two-way interactions. After model selection, the results of the best model are provided.

REPRODUCTIVE EFFORT, MOTHER BODY CONDITION AND OFFSPRING BODY CONDITION

The number of offspring in a litter (count data) is zero truncated and underdispersed (dispersion parameter = 0.26). Therefore, we computed reproductive effort (RE) as = litter size × mean offspring body mass^{0.75}/female postpartum body mass^{0.75} (Ebenhard 1990; Mappes *et al.* 2008), which can be used as dependent variable in linear models. RE is correlated with the number of offspring in the litter (Pearson's *r* = 0.89). Mother body condition was determined as individual residuals (standardized) from a linear regression model of postpartum body mass as predicted by the head width of the individual (Schulte-Hostedde *et al.* 2005). Offspring body condition was determined as individual residuals (standardized) from a linear regression of average offspring body mass at birth predicted by the average head width of the offspring in the litter at birth.

Mothers' reproductive effort (RE), mother body condition and offspring body condition were examined in relation to the mother's PUUV infection status using linear mixed models (LMM, lme function in nlme package in R software). In addition to the mother's PUUV infection status, the other explanatory variables in the RE models were the age of the animal (i.e. head width, centred value), the period of the breeding season (early or mid-BS), the phase of vole cycle (low, increase or peak) and all two-way interaction terms. In the mothers' body condition models, the explanatory variables were the mother's PUUV status, the period of the breeding season, the phase of vole cycle and the litter size and all two-way interaction terms. In the offspring body condition models, the initial explanatory variables were the mother's infection status and body condition, the litter size, period of the BS and the phase of the vole cycle and all two-way interaction terms.

MODEL SELECTION

After fitting each of the full models with all explanatory variables described above and a priori determined random effect *trapping session*trapping site*, a model selection for the fixed structure was

carried out. Model selection was based on Akaike Information Criterion corrected for small sample sizes (AICc) using 'dredge' function (library MuMIn) in R software: the model with fewest explanatory variables that lay within two AICc units of the model with the lowest AICc value was selected as the best model (Burnham & Anderson 2000). The binomial models (probability of breeding) were fitted using Laplace approximation method, and it was used also during the model selection procedure. Maximum likelihood (ML) method was used for fitting the models during the model selection of the linear mixed models. Restricted maximum likelihood (REML) method was used for estimating the parameter coefficients of the best models.

Results

Of the 890 female bank voles that were captured during the breeding season (May–August) over a nine-year study period (2002–2010), 266 females (29.9%) were gravid at the time of capture and gave birth. Probability of breeding was predicted by the period of the breeding season (BS) as well as the phase of the vole cycle, with highest likelihood of breeding in early BS and lowest likelihood in late BS, and with the most pronounced decrease during the peak phase and least in the low phase of the vole cycle (Table S2, Fig. S1). In early BS, most of the captured females (86%) gave birth, but PUUV-infection status was not selected in the best model and thus apparently not associated with breeding probability; note that no predictors (only intercept) were retained in the best model of breeding probability during early BS. In mid-BS, 54% of the females reproduced and now PUUV infection was associated with breeding probability through an interaction with age: at young age (small head width) the probability of breeding was higher in PUUV infected and at older age the probability of breeding was lower in PUUV infected in comparison with uninfected females (Table 1, Fig. 1). In late BS, only 5% of captured

females gave birth and probability of breeding was positively related with age (GLMM estimated effect (logit scale) for head width (\pm SE) = 5.446 (2.391), $z = 2.277$, $P = 0.023$), while PUUV infection status was not a statistically significant ($P = 0.160$) predictor of breeding.

Out of the 266 pregnant females, we obtained full data records for 246 litters (=1204 offspring) during the study period. Due to the few litters ($n = 21$) born to females captured in late BS and convergence problems with models, this season was excluded from the LMM analyses. Hence, the data set for analysis comprised 225 litters from 96 PUUV-infected and 129 PUUV-uninfected female bank voles. The litter size varied between one and nine offspring (mean = 5.35). Reproductive effort (RE) was not affected by female PUUV infection status (i.e. not selected in the best model), but it was affected by age (head width), with a positive association in early BS and negative association in mid-BS (Table 2); RE was also dependent on the phase of the rodent cycle, with significantly higher RE in increase phase than in low phase of the vole cycle (Table 2).

Puumala hantavirus infection status was associated with the mother's body condition: in early BS PUUV infected mothers were in worse condition than uninfected mothers, while in the mid-BS-infected mothers were in better condition than uninfected mothers (Table 3, Fig. 2). Offspring body condition was positively related to the body condition of the mother and negatively related to the number of offspring in the litter (Table 4). The mother's PUUV infection status, however, did not show direct relationship upon offspring body condition (Table 4).

Discussion

In this study, we examined the hypothesis that a chronic infection, caused by PUUV hantavirus, affects reproductive output of female host. Our data highlight the complex interactions that affect the breeding of females during the breeding season (BS). The probability of breeding (in the mid-BS) was related to the PUUV infection status and with the direction of this effect was affected by age: breeding probability was respectively higher and lower among young and old PUUV-infected individuals when compared with uninfected females. Moreover, postpartum body condition of mothers was related to PUUV infection status: in early BS PUUV-infected females were in worse condition and in mid-BS they were in better condition than uninfected females. Offspring body condition, in turn, was positively associated with the mother's body condition. At other times of the BS, PUUV apparently has no detectable association with reproductive capacity, and thus, we show clear seasonal effects to impact of PUUV infection on reproduction.

Higher breeding probability at young age among infected than uninfected individuals suggests that the infection enhances breeding in young females, which is in line with recent work showing that PUUV might increase the likeli-

Table 1. Female bank vole's likelihood of breeding (in logit scale) in relation to PUUV infection status, head width (centred value) and phase of the vole cycle (low, increase, peak) in mid-breeding season (BS). Intercept represents a PUUV-uninfected female with average head width that is captured in the early BS in low the phase of the vole cycle. σ^2 = the variance attributable to random effect. SD, standard deviation of σ^2

Source of variation	Estimate (SE)	<i>z</i> -value	<i>P</i> -value
Intercept	1.020 (0.642)	1.589	0.112
Head width	4.781 (0.757)	6.314	<0.001
PUUV infection	-0.077 (1.098)	-0.070	0.944
Cycle phase			
Increase	1.456 (0.789)	1.845	0.065
Peak	-1.557 (0.739)	-2.106	0.035
Head width * PUUV infection	-3.077 (1.005)	-3.062	0.002
PUUV infection * Cycle phase			
Increase	-1.932 (1.383)	-1.397	0.163
Peak	1.047 (1.080)	0.887	0.375
Random effect (site*session)	$\sigma^2 = 0.545$; SD = 0.738		

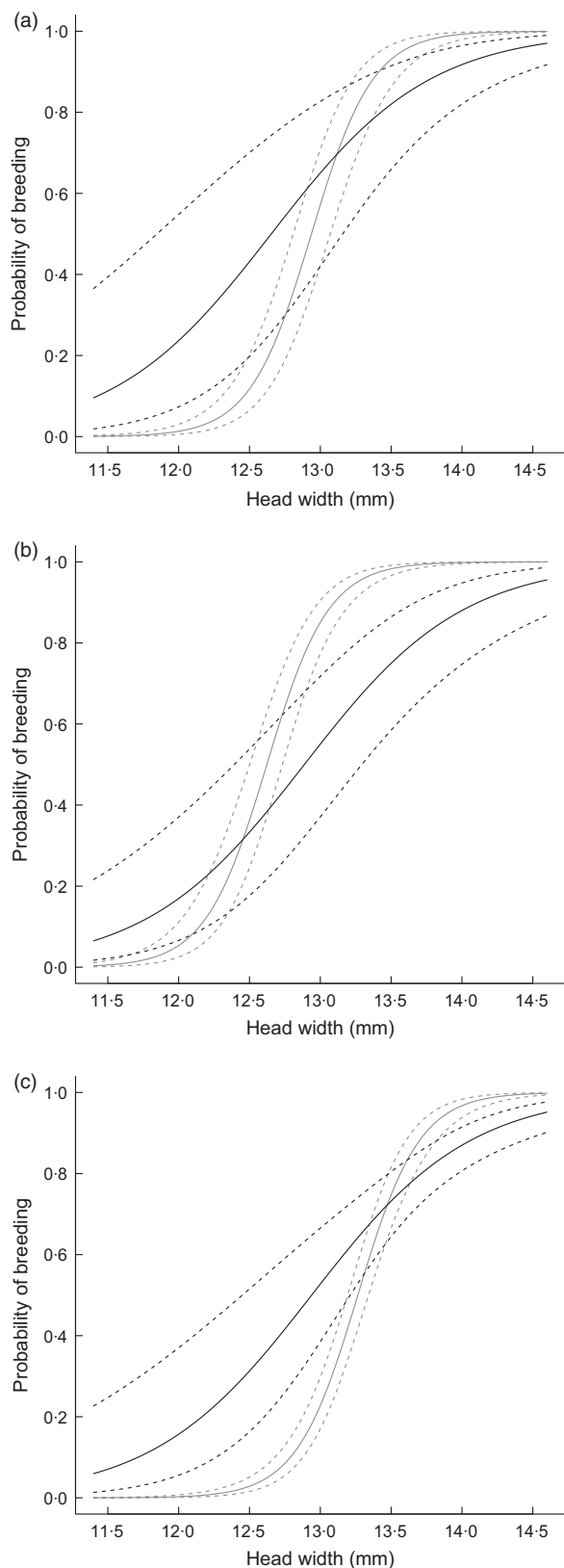


Fig. 1. Predicted probability of breeding (solid lines, \pm SE dashed lines) in relation to female's head width (proxy for age) and PUUV infection status (PUUV-infected = black lines, PUUV-uninfected = grey lines) in mid-breeding season in (a) low (b) increase and (c) in peak phase of the vole cycle. Predictions are based on GLMM (details in Table 1).

Table 2. Reproductive effort (RE) of the reproduced females in relation to the female's head width (centred value), period of the breeding season (early BS, mid-BS) and phase of the vole cycle (low, increase, peak). (Random = standard deviation of the random intercept (\pm residuals))

Source of variation	Estimate (SE)	d.f.	<i>t</i> -value	<i>P</i> -value
Intercept	0.697 (0.024)	161	28.752	<0.001
Head width	0.066 (0.031)	58	2.129	0.038
Mid-BS	0.008 (0.019)	161	0.387	0.699
Cycle phase				
Increase	0.118 (0.029)	161	4.047	<0.001
Peak	0.028 (0.027)	161	1.052	0.295
Head width*Mid-BS	-0.139 (0.041)	58	-3.376	0.001
Random effect (site*session)	0.037 (0.133)			

Table 3. The body condition of the mothers after giving birth in relation to PUUV infection status and to period of the breeding season (early and mid-BS) and the phase of vole cycle (low, increase and peak phase). Intercept represents a PUUV-uninfected mother that is captured in the early BS in the low phase of vole cycle. Random = standard deviation of the random intercept (\pm residuals)

Source of variation	Estimate (SE)	d.f.	<i>t</i> -value	<i>P</i> -value
Intercept	-0.302 (0.233)	159	-1.294	0.198
PUUV infection	-0.470 (0.197)	58	-2.382	0.021
Mid-BS	0.277 (0.334)	159	0.830	0.408
Phase of cycle				
Increase	-0.225 (0.300)	159	-0.750	0.455
Peak	0.478 (0.243)	159	1.972	0.050
PUUV infection	0.799 (0.266)	58	3.001	0.004
*Mid-BS				
Mid-BS *Increase	0.752 (0.404)	159	1.862	0.064
phase				
Mid-BS *Peak phase	-0.627 (0.348)	159	-1.802	0.073
Random effect (site*session)	0.189 (0.911)			

hood of reaching sexual maturity in bank voles (Tersago *et al.* 2012). These findings give support to the terminal investment theory (Clutton-Brock 1984; Mappes & Ylönen 1997), whereby with chronic infections that decrease future survival, and consequently future reproductive output, fitness may be maximized by allocating resources to current reproduction (e.g. Forbes 1993; Schwanz 2008b). As PUUV infection reduces bank vole's winter survival (Kallio *et al.* 2007), the likelihood of young infected individuals surviving until the following breeding season might be low, even if they allocate resources for (winter) survival rather than breeding during their first summer. Hence, allocating resources to breeding at a young age, even at the risk of reducing the long-term survival prospects, might be advantageous for infected individuals (Kaitala, Mappes & Ylönen 1997). While bank vole density is an important determinant of whether summer-born cohorts begin breeding during their first summer or delay breeding until the following season (Prévot-Julliard *et al.* 1999), our results

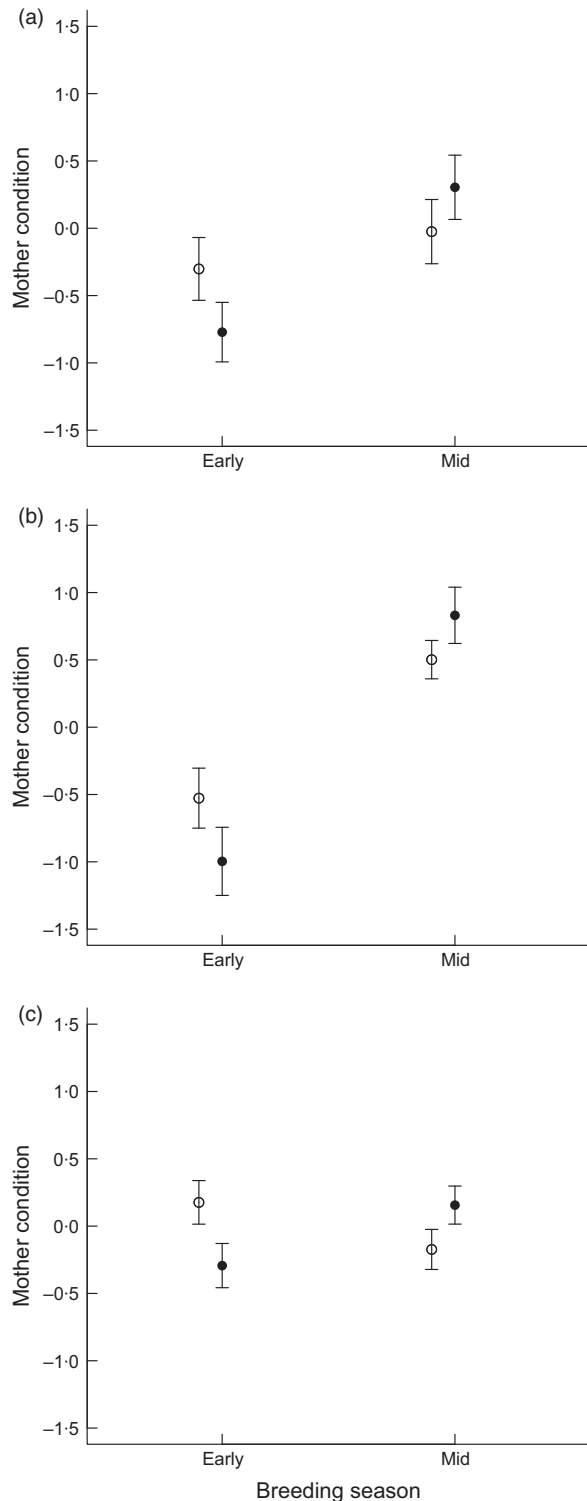


Fig. 2. Predicted mean mother condition (\pm SE) in relation to the animals PUUV infection status (open circle = PUUV-uninfected, solid circle = PUUV-infected) and period of the breeding season in (a) low, (b) increase and (c) peak phase of the vole cycle. Predictions are based on LMM (details in Table 3).

suggest that PUUV infection might impact individual decisions, with infected young individuals allocating resources for breeding.

Table 4. Offspring body condition (mean offspring weight of the litter at birth standardized by the mean head width) in relation to the mother's PUUV infection status and mother's body condition and litter size. Intercept represents the average offspring body condition of a mother that has average body condition. Random = standard deviation of the random intercept (\pm residuals)

Source of variation	Estimate (SE)	d.f.	<i>t</i> -value	<i>P</i> -value
Intercept	1.478 (0.293)	164	5.046	<0.001
Mother's body condition	0.209 (0.063)	58	3.318	0.002
Litter size	-0.276 (0.054)	58	-5.164	<0.001
Random effect (site*session)	0.108 (0.932)			

This infection-associated trade-off in resource allocation, however, interacts with the period of the breeding season and the age of the individual. In early BS, all females had over-wintered, and hence, they should allocate resources to current breeding (Kaitala, Mappes & Ylönen 1997); indeed, there was no statistical effect of PUUV infection upon the breeding probability in early BS. The offspring of the females that bred in early BS (i.e. individuals who were born in the early BS) either may start breeding during their first summer (in the mid-BS) or may delay breeding to the following summer. The mid-BS was the period of increased likelihood of breeding among young infected females compared with uninfected females. In the late BS, in turn, it is likely that only those females that have been breeding through the breeding season allocate their resources to reproduction for as long as possible as their survival to the following breeding season is unlikely (Prévot-Julliard *et al.* 1999), irrespectively of their infection status. Meanwhile summer-born individuals that have not yet reproduced/matured delay their breeding to the following breeding season (Prévot-Julliard *et al.* 1999).

Alternatively, a positive relationship between PUUV infection and reproduction among young individuals may occur when those individuals that start reproducing are more likely to acquire the infection due to hormonal and behavioural changes that increase their exposure and susceptibility to the infection (Escutenaire *et al.* 2002; Olsson *et al.* 2002; Tersago, Verhagen & Leirs 2011; Voutilainen 2013). While we tried to control for this effect by including individual age in the models, the order of events (maturity or infection first) among similar sized individuals cannot be completely taken into account with cross-sectional data. Consequently, we would like to emphasize that this alternative explanation is fully acceptable and in order to reject this hypothesis experimental or longitudinal capture-mark-recapture studies are required.

While the positive relationship between PUUV infection and the probability of breeding might be strategic and maximize the fitness of young infected animals, the observed negative effect of chronic PUUV infection among older females is likely to reflect the continued cost

of the infection. Indeed, the negative effect of PUUV infection on breeding probability reported here supports work that indicated that Sin Nombre hantavirus may impair the reproductive output of female host, the deer mouse (*Peromyscus maniculatus*), that have been infected for a long time (Dearing *et al.* 2009).

Puumala hantavirus-infected mothers were in worse body condition than uninfected mothers in early BS, when all of the reproduced individuals were old and had thus over-wintered (i.e. were born in the previous breeding season). In mid-BS, however, PUUV-infected mothers were in better condition than uninfected mothers. This may, at least partly, be linked to the finding that PUUV infection increased the breeding probability of young (small head width) and decreased the breeding probability of old (large head width) females (Fig. 1). In other words, the positive effect on mother condition may have resulted from age, rather than PUUV infection. The positive association between the body condition of the mother and the body condition of its offspring likely has important fitness consequences, as good body condition at birth may increase future breeding success of an offspring (Mappes & Koskela 2004; Oksanen *et al.* 2007; Schwanz 2008a). Hence, the poor body condition of over-wintered infected mothers may not only increase their susceptibility to further infections and further reduce their condition (Beldomenico & Begon 2010), but it may also determine the offspring condition and future fitness.

Any effect on the probability of breeding or body condition due to infections may have a pronounced effect on fitness at an individual level. Such individual-level effects imply that the infection transmission dynamics in host populations might be affected, for instance, if the proportion of offspring that infected- and uninfected-females produce is affected. As PUUV-infected females provide maternal antibodies (MatAb) to their offspring, which are transiently protected against the infection, the influx of susceptible individuals may be delayed if a large proportion of breeding females are infected and, consequently, the transmission of PUUV is delayed (Kallio *et al.* 2010, 2013). If PUUV infection reduces the breeding probability of territorial bank vole females, it enables the reproduction of uninfected females. Consequently, the proportion of maternally protected young bank voles may decrease with increasing proportion of susceptible young, which may facilitate the transmission of PUUV. Hence, the deleterious effect of an infection on females' reproductive output might increase the abundance of susceptible young individuals in the host population and hence be advantageous for the pathogen transmission and persistence. Meanwhile, positive effects on breeding probability may increase the proportion of maternally protected individuals and consequently, be disadvantageous for the pathogen transmission and persistence. While the role of maternal antibodies in disease dynamics in wildlife populations has been increasingly acknowledged (Gasparini *et al.* 2001; Staszewski *et al.* 2007; Boulinier & Staszewski

2008), the potential fecundity effects caused by the pathogen should be taken into account when the dynamics of maternal antibodies are modelled.

Recent studies indicate that endemic microparasites may regulate their rodent host population dynamics and even drive cyclic population abundance fluctuations (Soveri *et al.* 2000; Telfer *et al.* 2002, 2005; Burthe *et al.* 2006, 2008; Smith *et al.* 2008). However, as the deleterious effects of PUUV on the fecundity of bank vole are rather low and density independent (here independent of the cycle phase), it seems unlikely that the fecundity effects of PUUV would drive the cyclic population dynamics of bank voles in Northern Fennoscandia. Especially, as the cycles are characterized by synchronous crash of all sympatric species (Hansson & Henttonen 1988), a host-specific pathogen, such as PUUV, is not likely to contribute to all local vole species in a similar manner (Kallio *et al.* 2007). However, PUUV infection may contribute together with other factors, such as food and predators (Huitu *et al.* 2007; Korpela *et al.* 2014), on population level dynamics of the bank vole.

To summarize, our data predict that chronic pathogens can have adverse effect on the fitness of female bank voles as old-infected females have lower likelihood of breeding than similar aged uninfected individuals and as infected over-wintered mothers are in worse condition than are uninfected mothers. Moreover, our results predict that chronic infection can have positive effects on reproduction of young females, as young infected females have higher likelihood of breeding than similar aged uninfected individuals and as (young?) infected mothers are in better condition than uninfected (old?) mothers. While these effects are unlikely to determine alone the population dynamics of bank voles, they might have an influence on the abundance of susceptible young individuals in the host population, which may be translated into the transmission of the virus in the host population. Due to the complex nature of the associations between the infection, host age and period of the breeding season, the potential general effects of chronic infections on female hosts' reproduction should be further examined in other systems and taken into account when estimating the dynamics of pathogens in general. Our results highlight the complex nature of the interactions between a zoonotic pathogen and its reservoir rodent host, calling for further need to model these relationships in the dynamics of pathogen transmission in nature, which enable to predict the risks zoonotic pathogens pose to humans.

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Behavior Society Guidelines for the Use of Animals in Research, the legal requirements in Finland, and all institutional guidelines with permissions. The authors declare no conflict of interest.

Data accessibility

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.g4q84> (Kallio *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Model selection table for the probability of breeding.

Table S2. Female bank vole's probability of breeding in relation to the breeding season and the phase of the vole cycle.

Table S3. Model selection table for reproductive effort (RE), mother body condition and offspring body condition.

Fig. S1. Female bank vole's predicted probability of breeding in relation to the period of the breeding season and the phase of the vole cycle.