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Eva R. Kallio

Experimental Ecology on the Interaction between the Puumala Hantavirus and its Host, the Bank Vole

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ABSTRACT

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More than half of the known human pathogens have their origins in various animal species in nature. To understand the zoonotic risks for humans, the biology and relationships between a specific pathogen and its carrier host species need to be well known. In this thesis, the relationship between Puumala hantavirus (PUUV), and its carrier, the bank vole (*Myodes* [*Clethrionomys*] *glareolus*) was investigated using an experimental approach. Laboratory experiments showed that PUUV remains infectious outside the host for a prolonged period of time. This is influenced by the environmental conditions. The maternal antibodies that infected females provide to their progeny postponed the PUUV infection and enhanced the breeding success of young bank voles. The role of the infection status of breeding females in the transmission dynamics of PUUV in bank vole populations was studied using two experiments in nature. Accordingly, the infection status of breeding females has substantial influence on the transmission of PUUV to and among the young bank voles during and soon after the breeding season. PUUV infection had a negative influence on the over-winter survival of bank voles, whereas the breeding success or survival during the breeding season was not affected by the infection. These studies suggest that PUUV transmission in the bank vole populations is influenced by the temporary immunity the maternal antibodies provide, by the prolonged survival of PUUV outside the host and by the decreased winter survival of PUUV infected bank voles.

Key words: Bank vole; Infection prevalence; Maternal antibodies; Puumala hantavirus; Robo-virus; Transmission dynamics; Zoonotic diseases.

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II Kallio E. R., Poikonen A., Vaheri A., Vapalahti O., Henttonen H., Koskela E. & Mappes T. Maternal antibodies postpone hantavirus infection and enhance individual breeding success. Proceedings of Royal Society B 273, 2771-2776.

III Kallio E. R., Henttonen H., Vapalahti O., Koskela E. & Mappes T. Host sex and hantavirus transmission dynamics. Manuscript.

IV Kallio E. R., Voutilainen L., Vapalahti O., Vaheri A., Henttonen H., Koskela E. & Mappes T. Temporary immunity via maternal antibodies shapes hantavirus transmission in bank voles. Manuscript.

V Kallio E. R., Voutilainen L., Vapalahti O., Vaheri A., Henttonen H., Koskela E. & Mappes T. Endemic hantavirus infection impairs the winter survival of its host. Submitted manuscript.

1 INTRODUCTION

In the years following World War II, it was widely believed that man was going to win the centuries long war against infectious diseases using antibiotics and vaccinations. This optimism has proved premature, since infections, caused by more than 1400 known human pathogens, are killing millions of people annually still nowadays (www.who.int). More than a half of the known human pathogens are zoonotic, having their origin in various animal species in nature. Also, the majority of the emerging or the re-emerging pathogens are zoonotic (Taylor et al. 2001, Zeier et al. 2005, Woolhouse & Gowtage-Sequeria 2005). Rodents (order Rodentia) are the reservoir for many zoonoses. As the most specious and widely distributed mammalian group, characterized by a great deal of variation in ecology and life-history, rodents are assumed to maintain a considerable diversity of zoonotic pathogens, which are still to be found. For instance, hantaviruses represent a group of zoonotic rodent-borne (robo) viruses distributed in the world more widely than any other viruses carried by wildlife animals (Nemirov et al. 2004). Many rodent species live in close association with humans, and environmental changes, e.g. due to direct human activity and climate change, may further affect these contacts. Therefore, it is a great challenge to understand the features involved in the transmission of the zoonotic pathogens to humans. However, to evaluate the human risks the biology and the relationships between a specific pathogen and its carrier host species need to be well known (e.g. Mills & Childs 1998, Davis et al. 2005, Matthews & Woolhouse 2005). In this thesis, the relationship between a zoonotic Puumala hantavirus (PUUV), and its carrier, the bank vole (*Myodes* [*Clethrionomys*] *glareolus*) was studied. (Wilson & Reeder 2005 changed the genus name *Clethrionomys* to *Myodes*). PUUV represents a directly (without vector) and horizontally (not from mother to offspring) transmitted microparasite.

1.1 Microparasite-host interaction

The spread of a pathogen in the host population from one individual to another determines the fitness of the pathogen. The success of a microparasite is described with the term *basic reproductive rate* (R_0), which describes the number of new infections arising from one infected host (Anderson and May 1979, Anderson 1995, Heesterbeek & Roberts 1995, Hudson et al. 2002, Swinton et al. 2002). Thus, an infected individual has to create at least one new infection ($R_0 \geq 1$) to allow the microparasite to spread and persist in the host population. With directly transmitted viral infections, the R_0 is influenced by (i) the time period an infected individual is infectious, (ii) the availability of susceptible individuals in the host population, and (iii) by the transmission rate of the microparasite (β) (Andersson & May 1979, Anderson 1995, Heesterbeek & Roberts 1995, Swinton et al. 2002). The infectious period is influenced by the chronic or transient nature of the infection; with chronic infections the host is not capable of clearing the infection and it may remain infectious for life. The availability of susceptible individuals depends on the birth rate of the host. The transmission term (β) describes the efficiency of the pathogen to infect a susceptible host and depends on the likelihood of contacts between hosts and the probability of the transmission (Andersson & May 1979, Anderson 1995). Thus, the transmission term depends on the pathogen's ability to avoid the host's immune defense mechanisms and on the host's susceptibility for the infection, and is usually assumed to be constant for any given host-pathogen combination (Begon et al. 2002).

For epidemiological studies the host population of a microparasite is often classified as susceptible (S), infected (and infectious; I) and recovered or removed (R) individuals, so-called SIR-model (e.g. Anderson & May 1979). In the host population, the number of new infections arising during a period of time ($I_{(t, t+1)}$) depends on the availability of infectious (I) and susceptible (S) individuals, and on the transmission rate (β) of the microparasite (e.g Anderson & May 1979, Begon et al. 1998, Begon et al. 2002, Swinton et al. 2002). The pathogens that are transmitted directly and horizontally are assumed to create new infections in relation to the density of susceptible and infected individuals. There, the amount of contacts, and the amount of new infections, should increase with the host population density, and the transmission is said to be density-dependent. Consequently, there should be a host population density, i.e. a threshold density, below which the number of new infections does not replace the rate in which the infectious individuals are removed (e.g. Anderson & May 1979, Anderson 1995, De Jong et al. 1995, Begon et al. 1998, 2002, McCallum et al. 2001). Another transmission type, commonly discussed in literature, is the so-called frequency-dependent transmission. This type of transmission is assumed for sexually transmitted diseases and for vector -borne pathogens. There the number of e.g. sexual partners is not assumed to increase with population density and thus the spread of the pathogens depends on the

proportion of infected individuals in the host population (e.g. May & Anderson 1979, De Jong et al. 1995, Begon et al. 1998, 2002, McCallum et al. 2001, Ryder et al. 2005). In reality, however, no single type of transmission is usually valid to describe the observed patterns of infection dynamics in natural host populations (e.g. Begon et al. 1998, 1999, 2002, Fenton et al. 2001, Ryder et al. 2005).

In natural populations, there are differences between individuals in their susceptibility to the infection, based on genetic variability but also on gender, reproductive status and age, which may affect the host's likelihood to acquire and transmit the infection (Garnett & Holmes 1996, Woolhouse et al. 1997, Swinton et al. 2002, Wilson et al. 2002). The host demography and density usually change seasonally, which influences the parasite transmission and the course of the infection epidemics. Typically, high population densities facilitate the spread of a pathogen, whereas during low population density the extinction risk of a pathogen is high (May & Anderson 1979, Dye et al. 1995, Swinton et al. 2002, Sauvage et al. 2003). For a long-term persistence of a microparasite in the host population, the inflow of new susceptible host individuals needs to be high enough to support consistent transmission. With epidemic-causing pathogens (i.e. a rapid spread or increase in the prevalence of the pathogen (Watt at el. 1995)), the inflow of new susceptible individuals becomes often too low in comparison to the spread of the pathogen. This may be the result of extensive mortality or decreased fecundity which the infections often cause to their hosts. Consequently, the transmission rate decreases and the pathogen is at a risk of extinction from the host population (e.g. Swinton et al. 2002). When transmission rate and inflow of susceptible hosts enable consistent transmission and long-term persistence, a pathogen tends to be endemic in the host population. The endemic infections are often benign, causing no clinical illness. Although not causing obvious deleterious effects, the endemic pathogens may still be harmful for the host by decreasing the survival or the reproduction rate of the host (e.g. Anderson & May 1979, Dobson & Hudson 1995, Feore et al. 1997, Tompkins & Begon 1999, Telfer et al. 2002, 2005, Tompkins et al. 2002). This may be due to, for example, resource allocation between immune response and other fitness related traits (e.g. Sheldon & Verhulst 1996). These effects might be difficult to observe, especially in natural populations, because they may be confounded by other factors influencing fitness (Tompkins et al. 2002, Telfer et al. 2002, 2005). If the infection affects the host population dynamics, the transmission dynamics of the pathogen also depend on the pathogenic effects on the host. Therefore, determining the effects the pathogen induces on the hosts is a part of the biological information required to understand the transmission dynamics of microparasites (e.g. Matthews & Woolhouse 2005).

1.2 The aim of the thesis

The aim of this thesis is to study the interaction between zoonotic rodent-borne Puumala hantavirus (PUUV) and its carrier, the bank vole (*Myodes glareolus*) using both field and laboratory studies. The studies were conducted to improve the knowledge of the factors influencing the epizootic processes of PUUV, and features which may contribute to the transmission of rodent-borne (robo) viruses in general. The main aim of chapter (I) is to determine the longevity of PUUV outside the host. Specifically, this study deals with the knowledge of the transmission routes, the survival of PUUV outside the host and the environmental conditions influencing the longevity of PUUV. In three chapters of my thesis (II, III, IV) the scope is the maternal antibodies an infected mother transfers to its offspring. The period of the maternal protection and the timing of the infection as well as other fitness related effects the maternal antibodies offer to the offspring are studied in chapter (II)**.** The influence of the maternal antibodies on the spread of the infection in the host populations is studied in chapters (II), (III) and (IV). The effects of the PUUV infection on the reproduction and the survival of the bank vole are studied in chapters (II), (IV) and (V).

2 MATERIALS AND METHODS

2.1 Study species

PUUV belongs to hantavirus genus (family *Bunyaviridae*), and is carried by the bank vole (*Myodes glareolus*) (Brummer-Korvenkontio et al. 1980). Each hantavirus (more than 30 described species (Zeier et al. 2005)) has its own specific rodent host species, with whom they have co-evolved for millions of years (e.g. Nemirov et al. 2004). Hantaviruses occasionally infect ('spill-over') other species, including humans (e.g. Niklasson & LeDuc 1987, Niklasson et al. 1995, Escutenaire et al. 2000a, Klingström et al. 2002). In humans, PUUV causes *nephropathia epidemica*, a mild form of hemorrhagic fever with renal syndrome (HFRS) (Vapalahti et al. 2003). Secondary species, including humans, are not known to spread the PUUV further. The specific rodent host species represent the only evolutionary and ecological setting for each of the hantaviruses. The hantavirus infection does not cause clinical illness in the rodent host and the infection is chronic (Lee et al. 1981, Yanagihara et al. 1985, Childs et al. 1989, Gavrilovskaya et al. 1990, Hutchinson et al. 1998, Bernshtein et al. 1999, Botten et al. 2002, Meyer & Schmaljohn 2000, Compton et al. 2004). Hantaviruses are transmitted horizontally, via direct contacts or contaminated environment (e.g. Gavrilovskaya et al. 1990, Niklasson et al. 1995, Bernshtein et al. 1999, Botten et al. 2002). Thus, they are assumed to represent density-dependent transmission in the host populations (Mills et al. 1999).

The bank vole, the carrier of PUUV, is a common rodent species, characterized by a short life span and polygamous breeding behavior (Tkadlec & Zejda 1998). In Finland, the breeding season lasts from May to September, during which females give birth up to four litters with normally 4 to 8 pups per litter (Koivula et al. 2003). Mature females are territorial during the breeding season, whereas males have large home ranges (e.g. Koskela et al. 1997). Bank voles are able to mature soon after weaning (3-4 weeks old). However, usually only the individuals of the first cohort mature during the summer of birth; others delay breeding to the next year (Mappes et al. 1995, Tkadlec & Zejda

1998, Prévot-Julliard et al. 1999). In Northern Fennoscandia, bank vole populations show seasonal and multiannual fluctuations. The multiannual fluctuations in the densities of rodents, i.e. vole cycles, are characterized by a length of three to five years and a high, up to 100 fold, amplitude. The cycles are simultaneous with other sympatric vole species and synchronous over large areas, and are generally attributed to predation by specialist predators (reviewed e.g. by Hansson & Henttonen 1988, Hanski et al. 1991, 2001, Korpimäki et al. 2003).

2.2 Experimental procedures

The experimental procedures carried out in this thesis are reviewed in the Table 1, and detailed descriptions are given in each chapter.

The field studies (II, III, IV, V) were carried out in Konnevesi area in Central Finland (62° 37' N, 26° 20' E). The studies (II) and (III) were conducted in outdoor enclosures (each 0.2 ha), which enclose the study populations. The studies (IV) and (V) were carried out on 21 islands in Lake Konnevesi. The size of the islands varied from 0.24 to 3.20 hectares and the minimum distance from the mainland was 180 meters. The islands were covered by typical boreal forest, a natural habitat for bank voles. In all field studies, the bank voles were monitored using multiple-capture Ugglan Special live traps (Grahnab, Sweden). Besides the field experiments, the animals were studied in laboratory facilities at the Konnevesi Research Station (University of Jyväskylä). In the laboratory the rearing conditions and handling of the animals followed all institutional guidelines and animal experimentation permits and the legal requirements in Finland. Wood shavings and hay were provided as bedding; food (Labfor 36, Lactamin AB, Stockholm, Sweden) and water were provided *ad libitum*. The study (I) was entirely carried out in a laboratory environment. This animal experiment was carried out in the Swedish Institute for Infectious Disease Control (SMI), Stockholm, Sweden, where the guidelines of SMI in handling and rearing the animals were followed. The cell-culture -part of the study was conducted in the Haartman Institute of the University of Helsinki in Finland.

In the studies (I) and (III) the bank voles were inoculated subcutaneously with PUUV. In chapter (I) the virus strain was Kazan-wt and the dose was 100 times the infectious dose that successfully infects 50% of animals. The susceptible recipient individuals were exposed to the beddings of inoculated donors in the study (I). In order to prevent the introduction of a foreign strain of PUUV into the nature of Konnevesi area, the well-characterized Kazan-wt -strain was not used in the study (III). Therefore, the pool of lungs of six naturally PUUV infected (seropositive) old bank voles, trapped in Konnevesi area, were used as the infectious material. In the study (II) the study animals were exposed to the pool of beddings of naturally infected individuals. In the studies (IV) and (V) the infected individuals had acquired the infection

naturally in nature. In chapter (III), the immunization of bank voles against PUUV infection was done by subcutaneous inoculation of baculovirus-expressed recombinant PUUV nucleocapsid protein that has previously shown to protect bank voles from PUUV infection.

In the studies II, IV and V the infection status of bank voles was determined indirectly by detecting the presence of PUUV specific IgG antibodies in blood. The antibodies were detected using immunofluorescence assay (IFA) (II, IV, V) and enzyme immuno assay (EIA) (I and III). The avidity, i.e. the strength of the binding between the antibodies and antigen, was investigated in the study IV to determine whether seropositivity is a result of maternal antibodies or of an individual's own immune response. In the study (I), a more direct and sensitive method was possible to be used because the animals were sacrificed at the end of the study. RT-PCR method was used to detect viral RNA from lung samples of the sacrificed animals**.** To determine the longevity of cell-cultured hantaviruses (I), infected Vero E6 -cells were stained with IFA -method using PUUV antibody positive human sera.

TABLE 1 Simplified overview of the experiments, including study site, main aim of each study, the PUUV infection route and the characteristics of studied individuals.

Study no.	I	II	III	IV	V
Place	Laboratory	Enclosure	Enclosure	Island	Island
Main aim	Survival of PUUV outside the host	The influence of maternal antibodies on PUUV transmission and the fitness of bank voles	The influence of infectious host's gender on the transmission of PUUV to offspring	The role of females in the transmission of PUUV	The influence of PUUV infection to the survival of the host
Infection route	Virus inoculation / exposure to virus contaminated beddings	Exposure to virus contaminated beddings	Virus inoculation, immunization against infection	Natural	Natural

3 MAIN RESULTS AND DISCUSSION

3.1 Transmission of PUUV in bank voles (I, II, III, IV)

The hantavirus transmission is horizontal, via direct contacts, contaminated environment or aerosols. The infectious virus is shed in urine, feces and saliva (Lee et al. 1981, Yanagihara et al. 1985, Gavrilovskaya et al. 1990, Niklasson et al. 1995, Kariwa et al. 1998, Bernshtein et al. 1999, Meyer & Schmaljohn 2000, Botten et al. 2002, Padula et al. 2004). The study (I) shows that PUUV is transmitted via contaminated beddings for a prolonged period of time; PUUV remains infectious at room temperature for 12 to 15 days, and the duration of the infectiousness also depends on environmental conditions. Cold and humid conditions are favorable for the survival of the virus outside the host. For instance, at +4 °C PUUV remains infectious up to 18 days. Because no direct contacts between infected and susceptible bank voles were allowed, these results demonstrate also that hantavirus transmission does not require direct contact between rodents, a question that has been often discussed (Kariwa et al. 1998, Meyer & Schmaljohn 2000, Botten et al. 2002, Padula et al. 2004). Recently, Sauvage et al. (2003) suggested, using mathematical modeling, that an indirect mode of transmission via contaminated environment and prolonged virus survival outside the host is required to generate the observed epidemic patterns of PUUV in bank vole populations. Indirect transmission and prolonged survival outside the host makes the transmission of PUUV less dependent on the host density and enhances the persistence of the virus in the host population (Sauvage et al. 2003). Such persistence in the environment has also been speculated to play a role in the dynamics of Cowpox virus, another rodent-borne virus (Begon et al. 2003). Thus, the longevity in the environment might be a common adaptive characteristic of robo -viruses.

The transfer of antibodies from a mother to its progeny provides the offspring a passive transient immunity against the microbial infections the mother has encountered (Verhagen et al. 1986, Gavrilovskaya et al. 1990, Dohmae et al. 1993, Bernshtein et al. 1999, Borucki et al. 2000, Botten et al. 2002,

Grindstaff et al. 2003). Thus, the maternal antibodies may have a substantial, although temporary role in the susceptibility of young bank voles to PUUV infection. This was investigated in the study (II), where the offspring of PUUV-infected female bank voles were found to have a detectable level of the maternal antibodies until the age of 60 days. The temporary immunity lasted longer than the maternal antibodies were detectable. Some of the individuals that were not protected by the maternal antibodies were found to be infected by the age of 80 days, whereas no infections were detected in the offspring of infected females by this age (II). Thereafter, there was no difference in the infection prevalence between the MatAb+ and MatAb- individuals (II). Thus, the infection status of breeding females had influence, via the maternal antibodies, on the susceptibility of young bank voles to become infected.

The transmission of PUUV in relation to the temporary protection the infected females provide their offspring, was investigated also in the studies (III) and (IV). In these studies, the founder females introduced into the enclosures (III) or onto the islands (IV), were either PUUV infected or non-infected. These founder individuals were responsible for establishing the bank vole populations during the experiments. The PUUV infection risk of young bank voles was significantly lower when their mothers were infected than when they were non-infected and this was also observed at the bank vole population level (III, IV). When females were infected, their offspring were protected temporarily against the infection, which was seen as a lower risk to become infected during the early stage of their life. The gender of the infectious individuals, therefore, affected the transmission of PUUV among the next generation and the most feasible explanation is the effect of maternal antibodies (III). The PUUV prevalence of breeding females may, thus, determine the transmission rate in the population until autumn.

The longitudinal field studies on hantavirus dynamics in rodent populations show that the infection dynamics of hantaviruses vary greatly, depending on the specific hantavirus-host -system, the temporal and spatial aspects, and the host population dynamics (e.g. Verhagen et al. 1986, Gavrilovkaya et al. 1990, Niklasson et al. 1995, Abbott et al. 1999, Bernsthein et al. 1999, Calisher et al. 1999, Mills et al. 1999, Escutenaire et al. 2000b, Calisher et al. 2001). PUUV transmission dynamics in bank vole populations have been found to reflect both direct (Verhagen et al. 1986, Olsson et al. 2002) or delayed density dependence with and without critical threshold densities (Niklasson et al. 1995, Escutenaire et al. 2000b). In many studies the linear relationship between the hantavirus prevalence and the host density has been lacking (Boone et al. 1998, Cantoni et al. 2001, Olsson et al. 2005). Because the hantavirus infection probability increases with the age of the rodent hosts (e.g. Niklasson et al. 1995, Escutenaire et al. 2000b, 2002, Olsson et al 2002), the prevalence tends to decrease when newborns enter the host population. Due to this juvenile dilution effect (Mills et al. 1999, Davis et al 2005) the prevalence often decreases during the breeding season despite the increasing host density (Verhagen et al. 1986, Abbott et al. 1999). However, the positive correlation between prevalence and bank vole density may be lacking also in intra-

seasonally (e.g. Olsson *et al.* 2005, Kallio et al. in preparation), indicating that the transmission may be influenced by other means than the simple density-dependent relationship. One of the potential factors may be the maternal antibodies which were shown to have the potential to shape the PUUV transmission dynamics in bank vole populations during and soon after the breeding season in the studies (II), (III) and (IV).

3.2 The influences of PUUV infection on the bank voles (II, IV, V)

Traditionally, hantavirus infections have been thought to be harmless to their rodent hosts despite some evidence suggesting cellular level alterations (e.g. Gavrilovskaya et al. 1990, Compton et al. 2004, Netski et al. 1999). No clinical illness, increased mortality or reduced fecundity due to hantaviruses have been reported in the rodent hosts (e.g. Verhagen et al. 1986, Childs et al. 1989, Gavrilovskaya et al. 1990, Bernshtein et al. 1999, Netski et al. 1999, Compton et al. 2004, but see Calisher et al. 2005). The question whether PUUV has influence on the bank vole was revisited in the studies (II), (IV) and (V). The breeding success of bank voles in relation to PUUV infection was investigated in the studies (II) and (IV). In both studies, the litter sizes of PUUV infected and non-infected females were similar. In addition, the condition of offspring and the growth of the newborn bank voles were monitored (II) and no differences were found in relation to the PUUV infection status of the mothers. However, the maternal antibodies had substantial impacts on the offspring. The maternal antibodies enhanced the maturation and breeding in young bank voles (II). The female offspring of PUUV infected mothers reproduced more often than the other females. In males, the size of testicles was larger in the MatAb+ than in MatAb- individuals at the age of 50 days. Consequently, the maternal antibodies together with other factors may determine whether an individual starts breeding in the summer of birth, or delays it to the next breeding season. Our results highlight the significance of maternal antibodies on the breeding success of the carriers.

The over-winter survival of bank voles was influenced by PUUV infection (V). The PUUV infected individuals had significantly lower probability to survive from October to May than the non-infected individuals. This was seen also in the spring population sizes and densities, which were the lower the higher the autumn PUUV prevalence was. These results indicate that PUUV may regulate bank vole populations, but only during the winter season. Because no difference in the survival of infected and non-infected bank voles were found in the summer (IV), the deleterious effects on the over-wintering may be mediated by the harsh environmental conditions. However, the mechanisms causing the deleterious effects were not studied and they remain unknown so far. Thus, the regulating role of PUUV in bank vole populations

remains largely unknown. When considering the multispecies rodent cycles, the infection risk of the other rodent species and the effects of PUUV on them should be studied to evaluate the role of PUUV in vole cycles.

3.3 Implications for human epidemiology

Human *nephropathia epidemica* epidemics have been explained by rodent density: the higher the rodent density, the more human cases (e.g Brummer-Korvenkontio et al. 1982, Niklasson & LeDuc 1987, Niklasson et al. 1995, Vapalahti et al. 2003, Henttonen & Vaheri 2006). In Finland, for instance, depending on the geographic synchrony of bank vole cycles, ca. 750 - 2600 human cases have been diagnosed annually during the last decade (Henttonen & Vaheri 2006, www.ktl.fi). Although the bank vole density seem to explain the infection risk for humans, the predicted, by models (Davis et al 2005), positive correlation between PUUV prevalence and bank vole density is often lacking in autumns (Olsson et al 2005, Kallio et al. in preparation). It may be partly explained by the influence of maternal antibodies (II, III, IV). Maternal antibodies postpone the infection, and the proportion of maternally protected young individuals in a population depends on the infection prevalence of the breeding females. This may also explain why the peak of the human epidemics sometimes coincides the increasing phase of the vole cycles and sometimes the rodent peak densities (Henttonen et al. in preparation): high seroprevalence in early summer leads to high proportion of young with maternal antibodies, which delays the infection process later in the summer and autumn (III, IV) when voles come in contact with humans. In addition, the human infection risk may be influenced by the environmental conditions because of their influences on the infectivity of PUUV outside the host (I).

4 CONCLUSIONS

In this thesis I have studied the relationship between the PUUV and the bank vole at individual and population levels. The temporary protection the immunocompetent females provide to their offspring by maternal antibodies was shown to postpone the infection, shape the course of the infection epidemics, and influence the fitness of the host. Since maternal antibodies are a universal phenomenon among vertebrates, the role of maternal antibodies in the transmission dynamics of wildlife infections should be taken into consideration and further studied also in other microparasite - host systems. In addition, PUUV was proven to remain infectious outside the host for a prolonged period of time, suggesting that the survival of directly and horizontally transmitted pathogens outside the host should be further studied in other systems to clarify the general importance of the finding.

As a novel finding, I have also shown that PUUV infection has a negative effect on bank vole by decreasing its survival over winter. The mechanisms causing the decrease in the survival remain unknown, and thus the impact of hantavirus infection on the host individuals, as well as on the host population dynamics, should be further investigated.

To summarize, PUUV transmission in the bank vole populations may be influenced by the temporary immunity the maternal antibodies provide, by the prolonged survival of PUUV outside the host, and by the decreased survival of PUUV infected bank voles. The importance of these characteristics on the transmission dynamics of PUUV may vary with seasons. By taking these characteristics into account, more reliable models on the hantavirus transmission dynamics in the host populations and on the human epidemics may be constructed. To conclude, these findings may be crucial to understand the transmission dynamics of hantaviruses in general, and to predict the risk they may cause to humans.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Kokeellista ekologiaa Puumala-viruksen ja metsämyyrän välisestä vuorovaikutussuhteesta

Ihmisten infektiotaudeista yli puolet on zoonooseja eli ihmisiin tarttuvia eläinperäisiä tauteja. Jyrsijät toimivat useiden tällaisten ihmisiin tarttuvien taudinaiheuttajien, patogeenien, kantajina luonnossa. Näiden taudinaiheuttajien ihmiselle aiheuttaman epidemiologisen riskin ymmärtämiseksi ja ennustamiseksi on tunnettava patogeenin esiintyminen ja yleisyys isäntäpopulaatiossa sekä patogeenin esiintymiseen ja leviämiseen vaikuttavat ekologiset ja evolutiiviset tekijät. Väitöskirjatyössäni tutkin ihmisissä myyräkuumetta aiheuttavan Hantaviruksiin kuuluvan Puumala-viruksen ja sen isäntälajin metsämyyrän välistä vuorovaikutussuhdetta. Erityisesti tutkin Puumala-viruksen leviämiseen myyräpopulaatioissa vaikuttavia tekijöitä sekä infektion metsämyyrälle aiheuttamia vaikutuksia.

Hantavirusten tartuntatapa myyrästä toiseen ja toisaalta ihmiseen on ollut vilkkaan tutkimuksen ja keskustelun kohde. Väitöskirjatyössäni tutkin Puumala-viruksen infektiokyvyn säilymistä isännän ulkopuolella ja vallitsevien olosuhteiden vaikutusta tartuntakyvyn kestoon. Huoneenlämmössä virus säilyi tartuntakykyisenä myyrille noin kahden viikon ajan, ja kylmä ja kostea ympäristö paransi entisestään viruksen infektiokyvyn säilymistä. Lisäksi koe osoitti, etteivät suorat kontaktit ole edellytys viruksen leviämiseksi myyrien välillä tai myyristä ihmiseen. Puumala-viruksen tartuntakyvyn pitkä säilyminen isännän ulkopuolella tehostaa viruksen leviämistä. Se myös edistää viruksen säilymistä alueellisesti metsämyyrätiheyden ollessa ajoittain alhainen.

Metsämyyrän alttius infektoitua riippuu esimerkiksi perinnöllisistä tekijöistä, yksilön iästä, sukupuolesta ja lisääntymistilasta. Infektioalttiuteen vaikuttavat myös maternaaliset vasta-aineet. Näillä tarkoitetaan infektoituneen naaraan tuottamia vasta-aineita, jotka siirtyvät istukan ja/tai maidon välityksellä emolta jälkeläisille. Väitöskirjatyössäni osoitin, että maternaaliset vasta-aineet suojaavat nuoria metsämyyriä Puumala-virusta vastaan noin kolmen kuukauden ikään asti. Siten infektoituneen naaraan jälkeläiset altistuvat infektiolle vanhemmalla iällä kuin suojaamattomat nuoret metsämyyrät populaatiossa. Maternaalisten vasta-aineiden tarjoaman suojan vaikutus oli nähtävissä myös populaatiotasolla. Kun virusta kantavien naaraiden osuus populaatiossa oli lisääntymiskauden alussa korkea, oli infektio jälkeläisten keskuudessa syksyllä harvinaisempi verrattuna populaatioihin, joiden naaraat eivät olleet keväällä infektoituneet. Lisäksi maternaalisten vasta-aineiden suojaamat nuoret metsämyyrät saavuttivat lisääntymistilan nuorempina kuin suojaamattomat yksilöt. Maternaalisilla vasta-aineilla oli siis yksilön lisääntymismenestystä parantava vaikutus.

Hantavirus -infektiot ovat isäntälajeissaan oireettomia eikä hantavirusten ole aikaisemmin havaittu tai oletettu aiheuttavan haittavaikutuksia isännälleen. Väitöskirjassani tutkin tätä oletusta uudelleen vertailemalla tartunnan saaneiden metsämyyrien lisääntymismenestystä ja elossasäilymistä suhteessa infektoitumattomiin yksilöihin. Tartunnan saaneiden naaraiden poikasten lukumäärä, koko tai kunto eivät eronneet infektoitumattomien naaraiden poikasiin verrattuna. Puumala-virusinfektiolla en havainnut olevan vaikutusta metsämyyrän selviytymiseen lisääntymiskaudella. Sen sijaan infektiolla oli selvä negatiivinen vaikutus metsämyyrän selviytymistodennäköisyyteen talven yli. Infektoituneet yksilöt selviytyivät talven yli 2-4 kertaa huonommin kuin infektoitumattomat yksilöt. Tämä on ensimmäinen kerta, kun Hantavirus-infektion haitallinen vaikutus isäntälajinsa kelpoisuuteen on osoitettu.

Suomessa on diagnosoitu viimeisen kymmenen vuoden aikana 750-2600 Puumala-viruksen ihmisille aiheuttamaa myyräkuumetapausta vuosittain. Ihmisepidemioiden on katsottu riippuvan lähinnä myyräsyklin vaiheesta myyrätiheyden ollessa tärkein tekijä ihmistapausten ilmentymisessä. Suorat havainnot ihmistapausten määrän ja myyrätiheyden välisestä suhteesta kuitenkin osoittavat, ettei metsämyyrien tiheys yksin selitä myyräkuume-epidemioita. Osoittamani Puumala-viruksen pitkäaikainen säilyminen isännän ulkopuolella, etenkin kylmissä olosuhteissa, edistää viruksen yleisyyttä metsämyyräpopulaatioissa. Tämä voi osaltaan selittää usein loppusyksyyn ja alkutalveen ajoittuvia myyräkuume-epidemioita. Myös maternaalisten vasta-aineiden aiheuttama viive nuorten metsämyyrien infektioalttiudessa voi heijastua ihmistapausten määrään ja siihen, mihin ajankohtaan ihmistapaukset pääsääntöisesti ajoittuvat. Maternaalisten vasta-aineiden vaikutuksesta epidemian huippu voi siirtyä myöhäisemmäksi talveen. Siten väitöskirjatyössäni osoittamani Puumala-viruksen ja metsämyyrän välisen vuorovaikutussuhteen piirteet voivat osaltaan vaikuttaa ihmisten myyräkuume-epidemioiden ilmentymiseen. Uuden tiedon avulla pystytään myös yhä luotettavammin mallintamaan ja ennustamaan epidemioiden esiintymistä ja voimakkuutta niin myyrillä kuin ihmisilläkin.

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