**Scientific Note**

**Experimental investigation of a hantavirus host-switch between arvicoline rodents**

*Lemmus lemmus* and *Myodes glareolus*

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Virus host-switches have resulted in the emergence of numerous epidemic (including HIV, Wolfe et al. 2007) and epizootic diseases (such as Influenza A virus, Taubenberger and Kash 2010). Spillover is an obligatory first step to a pathogen host-switch (Parish et al. 2008), which is believed to occur far more frequently than sustained transmission within the novel host species (host-switch). Encounter opportunities increase the likelihood of spillover, while virus replication fidelity and the phylogenic relationship between host species may aid in cell attachment and the evasion of host defenses (Parish et al. 2008). Here we use the context of a World War II epidemic to investigate a potential hantavirus host-switch from bank voles (*Myodes glareolus*) to Norway lemmings (*Lemmus lemmus*). Hantaviruses are an emerging disease family for which the number of species and hosts has risen markedly in recent years, along with the identification of spillover and host-switches (Ramsden et al. 2009, Guo et al. 2013).

During World War II, approximately 10,000 German and Finnish soldiers stationed in Finnish Lapland suffered a disease resembling nephropathia epidemica (NE) (Stuhlthauft 1943, Hortling 1946, Clement et al. 1997), a mild form of haemorrhagic fever with renal syndrome (HFRS), caused by Puumala hantavirus (PUUV) (Vapalahti et al. 2003, Vaheri et al. 2013). Reported symptoms included high fever, renal insufficiency, and occasionally myopia, with recovery in approximately two weeks (Stuhlthauft 1943, Hortling 1946). This number of cases and combination of symptoms, particularly with the pathognomonic feature of myopia occurring in 10–20% of patients, cannot be explained by any other currently known pathogen in the area. Veterans from this epidemic still carry antibodies suggestive of a hantavirus infection (Vapalahti et al. 1999). However, inferences are confounded by difficulties in locating previously infected soldiers and high serological prevalence in the wider population.

PUUV is the dominant hantavirus species in Fennoscandia (Vapalahti et al. 2003). Its reservoir host is the bank vole, and transmission to conspecifics and humans occurs primarily through the inhalation of infectious aerosolized excreta (Hardestam et al. 2008, Vapalahti et al. 2010). In Finland, 1,000 to 3,500 people are diagnosed annually with NE, generally in early to mid-winter when voles are most likely to enter human dwellings (Vapalahti et al. 2003).

The Lapland wartime epidemic occurred atypically in late spring and coincided with a marked density peak and migration of Norway lemmings (Vapalahti et al. 1999). This gives rise to two primary hypotheses regarding a lemming source of infection. Firstly, the epidemic may have been caused by a *L. lemmus* specific hantavirus, with symptoms similar to those of NE. However, no such virus has to date been identified in over 500 individuals screened from northern Finland and southern Norway (Vapalahti et al. 1999, later unpubl. material). Alternatively, the epidemic might indeed have been caused by PUUV - carried not by bank voles, but by Norway lemmings. While hantaviruses are considered mostly host specific, examples of spillover (Klingstrom et al. 2002, Schlegel et al. 2009) and host-switches have been documented in their evolution, including genera in Arvicolinae (voles and lemmings) (Vapalahti et al. 1999, Plyusnina et al. 2008). The migration of lemmings from their endemic habitats in the subarctic tundra into bank vole habitats of the taiga presents ample opportunities for sympatric interactions that may have facilitated PUUV spillover. No research has previously investigated whether PUUV can be transmitted from bank voles to lemmings. We report on an experiment to assess whether the excreta of PUUV-infected *M. glareolus* can successfully infect *L. lemmus* via natural transmission routes in a laboratory environment.

In September, 2010, 50 *L. lemmus* were captured by hand at Kilpisjärvi (69°02′57″ N, 20°47′40″ E) in north-westernmost Finnish Lapland and transported to the Suonenjoki rodent laboratory of the Finnish Forest Research Institute. Prior to experimental treatments, all lemmings were tested twice with an interval of one month for hantavirus antibodies. All antibody tests were conducted on blood taken from the retro-orbital sinus and analyzed with a commercially available rapid test (Ab-Dect Puumala, Reagena, Toivola, Finland).

Immediately prior to experimental treatments, *M. glareolus* were captured from forests at Suonenjoki (62°37′30″ N, 27°7′30″ E) and taken to the rodent laboratory. All voles were tested for PUUV antibodies and placed into cages composed of three seropositive and two seronegative individuals, or vice versa (five cages total). Experimentally infected bank voles have been found to shed PUUV RNA in...
their urine for up to 44 days post-infection (Hardestam et al. 2008), while studies with naturally infected wild voles have identified persistent shedding (Gavrilovskaya et al. 1990, Bernshtein et al. 1999). Similarly, our unpublished results show that approximately 50% of naturally infected wild voles are shedding virus at any time throughout their lifetime. This knowledge, in conjunction with serostatus, was used to infer shedding from experimental animals. It cannot be expected that all voles were continuously shedding virus or that some originally seronegative individuals were not already infected but had not yet seroconverted. Therefore, we employed diverse and repeated exposure methods over several months while utilizing excreta from all seropositive individuals, including those that seroconverted throughout the experiment. PUUV naïve voles were tested regularly to verify that infection/seroconversion had occurred and identify further donor individuals.

Three treatments were employed to infect lemmings: (1) Peat and bedding materials were collected from all vole cages, mixed, and dispersed into lemming cages (50 lemmings, two to three individuals per cage) twice per week for four weeks (see Gavrilovskaya et al. 1990). PUUV antibody tests were conducted four weeks after treatment termination. (2) Ten lemmings from the above 50 were randomly selected after treatment 1 and placed into the cages used to house voles for the preceding three to eight days. Lemmings remained in these cages for a minimum of three days, a method that has proved successful for PUUV transmission between bank voles (Kallio et al. 2006). The process was repeated three times with the same individuals, using different vole-soiled cages. Lemmings were bled and sacrificed eight weeks after treatment termination. (3) Lastly, 50µl of urine, pooled from >six voles (including voles that were originally seropositive and those that seroconverted throughout the experiment), was pipetted onto the nostrils of another ten lemmings randomly selected from the original 50 (see Hardestam et al. 2008). The treatment was repeated three to four times with the same individuals, at two to eight-day intervals. Lemmings were bled and sacrificed eight weeks after treatment termination.

PUUV antibodies, antigen, and RNA (see Plyusnin et al. 1997 for methods) were tested from terminal samples, including individuals that died at any stage of the experiment. All experimental methods were approved by the Finnish Animal Ethics Council (permit no. ESAVI-2010-08976/Ym-23).

Several (>five) of the originally PUUV seronegative bank voles seroconverted throughout the experiment. However, all lemmings remained seronegative despite our transmission efforts. Viral antigen and RNA were also absent from terminal samples. These results demonstrate that PUUV transmission from M. glareolus to L. lemmus via infectious aerosolized excreta is highly unlikely.

The experiment was designed to mimic modes of transmission that could be expected between sympatric vole and lemming populations. The most likely natural exposure scenario would result indirectly, via contact with virus containing excreta. Voles and lemmings occupy similar habitats and utilize the same runways, burrows and shelter areas in forests when their ranges overlap during a lemming migration (Henttonen et al. 1977, Henttonen and Kaikusalo 1993). Importantly, infectious PUUV is able to survive extended periods outside the host (12-15 days at room temperature (Kallio et al. 2006) and probably considerably longer in cold and damp conditions), making direct contact between bank voles and lemmings unnecessary for interspecific transmission.

Norway lemmings are competitively dominant over bank voles and very aggressive during their extensive migrations (Henttonen et al. 1977). This suggests that, although bank voles also shed PUUV RNA in saliva (Hardestam et al. 2008), they avoid direct encounters with lemmings and instead flee, and therefore would rarely have the opportunity to infect Norway lemmings by biting. Based on this, experimental inoculation of PUUV into lemmings, regardless of its potential efficacy as a transmission route, was dismissed as an experimental treatment. As such, we did not demonstrate that lemmings are incapable of supporting PUUV, but rather that its acquisition from bank voles via natural routes is highly unlikely. By contrast, aerosolized transmission between bank voles occurs relatively uninhibited, including between cages up to 1.5m apart (Gavrilovskaya et al. 1990, Bernshtein et al. 1999).

Several lines of evidence, namely the disease symptoms and the atypical timing of the epidemic, which coincided with a lemming peak and migration, support a hypothesis for the source of infection – hantavirus transmitted by Norway lemmings. Indeed, this outbreak, during which lemmings were described to run in the trenches, has even been referred to as ‘lemming fever’ (Stuhlfauth 1943). However, our results strongly suggest that PUUV cannot be naturally transmitted from bank voles to Norway lemmings, and by extension, lemmings were not infecting soldiers with PUUV. While a hantavirus, Topografov (TOPV), has been isolated from Siberian lemmings (Lemmus sibiricus), and been shown experimentally capable of infecting L. lemmus (Vapalahti et al. 1999), no natural hantavirus in has been found in L. lemmus. Unfortunately, the hantavirus antibodies that veterans from the epidemic still carry are too old for a cross-neutralization test to distinguish between PUUV and TOPV (Vapalahti et al. 1999).

After all, the epidemic may in fact result from bank vole-borne PUUV, even though it occurred unusually in spring, when PUUV-infected bank voles generally occur at low densities. War often requires soldiers to lodge in simpler dwellings that may increase exposure to rodents. Moreover, the presence of bank voles may have gone comparatively unnoticed when surrounded by the more conspicuous lemmings, and their effect amplified by the cool and wet conditions typical for the season in Lapland, which are suspected to enhance the stability of hantavirus infectivity once outside the host.

In conclusion, our results demonstrate that a bank vole to lemming host-switch of PUUV is a highly unlikely primary driver of the documented World War II epidemic. More generally, further experimental research on potential interspecific transmission of hantaviruses is clearly needed.
Climate change will alter species distribution ranges, and new species combinations will emerge. Spillover is often found in nature, yet adaptation into new species and the role of secondary species in transmission remains very poorly understood.

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