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The screening of parasites and viral pathogens of small mammals from a farm in southern Finland, and genetic identification of the Finnish house mouse, *Mus musculus*

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Seven species of small mammals ($N = 160$) caught on a cattle farm in southern Finland were screened for various parasites and viral pathogens. Antibodies to lymphocytic choriomeningitis virus were detected in 3.6% of *Mus musculus* ($N = 110$) and in 7.7% of *Apodemus flavicollis* ($N = 26$). Two (33%) *Myodes (Clethrionomys) glareolus* ($N = 6$) had Puumala virus antibodies, and one (11%) *Microtus agrestis* ($N = 9$) tested positive for cowpox virus. Of fungal organisms, *Pneumocystis* sp. (7.3%) and *Emmonsia parvum* (0.9 %) were found in histological examination of lung tissue of the house mouse. No blood parasites were detected in thin blood smears but kidney forms of *Trypanosoma musculi* were visible in impression smears of two of 27 (7.4%) house mice examined for the kidney forms. Meront forms of *Hepatozoon* sp. were detected in lung tissue sections in one *Myodes glareolus*. Of possible vectors of blood parasites, six species of fleas were recovered from the small mammals. House mice from the cattle farm had the sex chromosomes of the *M. m. musculus* type whereas mtDNA was of the *M. m. domesticus* type. House mice from another population in western Finland had both the nuclear and mitochondrial genome of *M. m. musculus*.

Introduction

Although commensal rodents are known to have a high potential to transmit a wide range of pathogens to humans or livestock (Pocock *et al*. 2001), information on the disease agents in common commensal such as that of the house mouse in Finland is scarce (Rislakki & Vasenius 1970). One reason for this is the fact that in Finland the number of house mice has decreased considerable during the last decades, mainly due to significant decline in the number of farms with dairy cattle and henhouses. In higher latitudes house mice are, however, especially closely associated with human dwellings because they compete poorly with the native rodents in fields and forests during severe winters. Despite their limited movements, house mice remain in contact with the native rodents because many of the latter species willingly invade buildings during times of harsh weather and low food resources in late fall and winter.

The only arenavirus presently described from Europe is the lymphocytic choriomeningitis virus (LCMV). Its main reservoir is believed to be the house mouse (Oldstone 2002). Our recent findings of arenavirus antibodies in many native rodent species in Finland (Laakkonen *et al*. 2006a) and elsewhere (Kallio-Kokko *et al*. 2006, Laakkonen *et al*. 2006b) warranted studies to determine whether these are spill-over from the LCMV carried by the house mouse, or represent new viruses specific to the particular rodent hosts. For this purpose, we screened house mice and native small mammals from a cattle farm for the presence of antibodies to arenaviruses. The animals were also screened for the presense of antibodies to other zoonotic viruses, namely cowpox- and hantaviruses. As an added benefit to better understand the epidemiology of rodent-borne zoonoses, we also studied the parasite diversity of these animals.

Since invasive animals like house mice, tend to carry only a subset of their potential disease agents during each colonization event (Fromont *et al*. 2001), information on the origin of an invasive species is important when assessing the role of the species as a carrier of emerging pathogens. Thus, we also subjected house mice from three different localities to molecular analyses to gain information on genetic variation among house mouse populations in Finland. It has been hypothesized that Scandinavia was colonized by a few individuals from the *musculus*/*domesticus* hybrid zone region near Kiel Bay in northern Holstein (Prager *et al*. 1993). The mice were genetically a mixture of *musculus* nuclear and *domesticus* mitochondrial genomes (Prager *et al*. 1993). The phenomenon of mice having nuclear genome of *Mus musculus musculus* and mitochondrial DNA of the *M. m. domesticus* type is well known from Sweden but it is documented only from few places in Finland (Prager *et al*. 1993).

Materials and methods

We snap-trapped the animals at the Suitia research farm (60°01´25´´N, 24°11´54´´E) of the University of Helsinki, located in southern Finland. The farm, which arable land and patches of mixed forests surround, has about 80 milking cows. Several farms are located within a kilometre of the farm, and the distance to the nearest town is 13 km. Trapping was done in October and December 2004, in September and December 2005, and in March 2006. Traps baited with cheese and/or dog chocolate were placed inside and outside the barns, nearby storage buildings, and to the vicinity of the buildings along the edge of a grazing field. About 100 snap traps were placed during each trapping period close to signs of small mammal activity. Due to the possible danger to the farm animals and machinery, traps could not be placed in a grid. Trapping lasted one or two days depending on the number of animals present, and traps were checked at irregular intervals depending on the mouse activity. The house mouse population of the farm was controlled with poison boxes operated by a professional rodent exterminator. In December 2005, the control effort was intensified because of the increased mice activity leading to a crash in mice densities in spring 2006. Because of the irregular placement of traps, and the use of poison, no quantitative estimate of the mouse population size (trapping index) was attempted.

In an effort to gain information on the distribution of LCMV of house mouse in Finland, we included virus screenings (*see* below) of this

species caught from four other sites (Alavus N = 1, and Ulvila N = 3 in western Finland; Uurainen N = 2 in central Finland; Parikkala N = 1 in eastern Finland) during our annual rodent monitoring (Laakkonen *et al*. 2006a).

At necropsy, we recorded the species, sex, and reproductive condition of the animals, and examined the animals macroscopically for parasites and anomalies. Any ectoparasites recovered from the animals were stored in 70% alcohol, and later shipped to Michael Hastriter (Brigham Young University, Provo, UT) for identification of fleas. Pathogens were screened with the use of microscopy or antibody detection.

For serological analysis, we placed the heart of each animal caught in a sterile vial containing 100 μl phosphate-buffered saline (PBS) and stored them at –20 °C until the diluted blood (1:10) was analyzed by immunofluorescence assay (IFA). We tested the reactivity of the blood samples to arenaviruses with LCMV-IFA, to cowpox virus (CPXV) with CPXV-IFA; and to hantaviruses with either Saaremaa virus (SAAV)-IFA for mice, or with Puumala virus (PUUV)-IFA for voles and shrews. The slides were prepared, and the IFA carried out as in Kallio-Kokko *et al*. (2005).

Pieces of lung, spleen and kidney of all animals caught were stored in RNALater™ for PCR analyses at –20 °C. We run arenavirus-PCR on all samples as described previously (Laakkonen *et al*. 2006b).

To detect blood parasites, we obtained a drop of blood from the heart of all animals caught for preparation of a thin blood smear, which was air-dried, fixed in ethanol, and stained with Giemsa's stain. Each smear was microscoped for 5 min at ×400 and for 10 min at ×1000. We prepared impression smears of kidney tissue from the 27 house mice caught during the first trapping period and microscoped (10 min at ×400) for kidney forms of *Trypanosoma musculi*.

We fixed pieces of lungs of all animals in 10% neutral-buffered formalin for the preparation of standard histological sections. These were stained with hematoxylin–eosin for screening of abnormalities in the lung tissue, and Gomori's Methenamine Silver (GMS) stain for detection of pulmonary fungi (Grocott 1955). Fecal samples (< 0.05 g) from the rectums were stored in 2.5% aqueous potassium dichromate ($K_2Cr_2O_7$) for analyses of *Eimeria* spp. parasites. After allowing any oocysts to sporulate at room temperature, the suspension was centrifuged for 3 min at 250 *g* and resuspended in a saturated magnesium sulfate ($MgSO_4$) flotation solution after which the sample was examined for oocysts by using a McMaster counting chamber.

For studies on the genetic variation of the house mouse, we analyzed the systematic status of the house mouse from the Suitia farm, and from western (Ulvila), and eastern (Parikkala) part of Finland trapped in a previous study (Laakkonen *et al*. 2006a), with both mitochondrial and sex-chromosome markers (no genetic material was available from Alavus and Uurainen). We examined mitochondrial DNA through sequencing two segments encompassing variable domains of the control region and flanking tRNA genes (N = 14) as described in Prager *et al*. (1993, 1996) and Macholán *et al*. (unpubl.). We characterized the X chromosome using two B1-type SINE insertions, one in the *Btk* gene (N = 10) and the second one in the *Tsx* gene (N = 2) which are present in *M. m. domesticus* and absent in *M. m. musculus*. The Y was typed with an 18-bp deletion within the last exon of the *Zfy2* gene which is fixed in *musculus* and absent in *domesticus* (N = 5; for details, *see* Munclinger *et al*. 2002, 2003 and references therein; M. Macholán unpubl. data).

Results

We caught a total of 160 small mammals of seven species (Table 1) at the Suitia farm. The animals appeared normal on gross pathologic examination except for the green and blue color patches seen on stomachs and intestines (N = 3), and pale spots on livers (N = 2) found in house mice. These probably resulted from eating the poison baits.

We found arenavirus antibodies in four (3.6%) *Mus musculus* and two (7.7%) *Apodemus flavicollis* (Table 1) trapped in Suitia. Two (33%) *Myodes (Clethrionomys) glareolus* had Puumala antibodies, and one (11%) *Microtus agrestis* tested positive for CPXV. The arenavirus-PCR attempts remained negative.

Of the seven house mice caught at other sites, one (from Alavus) tested positive for CPXV (Table 1). The number of house mouse caught in these sites was low partly because the regular rodent monitoring targets field and forest habitats (Laakkonen *et al*. 2006a). All these house mice came from field edges close to buildings.

In histological examination of the lung tissue, we detected fungal organisms closely resembling cysts forms of *Pneumocystis* sp. in the lungs of eight (7.3%) house mice. Five of these were females, and two were mature, reproducing animals. Of other pulmonary fungi, we found one adiaspore of *Emmonsia parvum* in one mature male house mouse. No *Eimeria* oocysts or eggs of intestinal worms were found in fecal samples.

We did not find any blood parasite forms in the thin blood smears (N = 160) of any of the small mammal species. We detected kidney forms of *Trypanosoma musculi* in the impression smears of 2 (7.4%) of the 27 house mice examined. In the impression smears of each infected house mouse, two *T. musculi* were visible. We found meront forms of *Hepatozoon* sp. in lung tissue sections in one immature male bank vole, *Myodes glareolus*. The fleas found in house mice and other small mammals are shown in Table 2.

Mice from Suitia and Parikkala had the sex chromosomes of the *M. m. musculus* type whereas mtDNA was of the *M. m. domesticus* type. The two mice from Ulvila had both the nuclear and mitochondrial genome of *M. m. musculus*.

Discussion

There are no previous studies on LCMV of wild house mice from Finland. The LCMV prevalence (3.6%) found in house mice examined in this study was at the lower end of the range detected in *Mus* spp. elsewhere in Europe (3.6% to 11.7%, Lledó *et al*. 2003), and outside Europe (2.5% to 9%; Morita *et al*. 1991, Child *et al*. 1992). Previous studies (Childs *et al*. 1992, Pocock *et al*. 2004) indicate that antibody screening presumably underestimates the overall infection, and that there might be significant differences in LCMV infection among locations because dispersal between territorial subgroups is known to be limited in commensal house mouse populations. The prevalence (7.7%) in *A. flavicollis* was similar to those found in this host species in our previous study from central Finland (6%; Laakkonen *et al*. 2006a) and from northern Italy (6.1%; Kallio-Kokko *et al*. 2006).

Our recent documentation of arenavirus antibodies in many wild rodent species collected from Finland, Italy, Croatia, Kazakstan and Turkey (Kallio-Kokko *et al*. 2006, Laakkonen *et al*. 2006a, 2006b, H. Henttonen unpubl. data), raises the question on the virus species involved, and nature of these infections. Due to the negative results in the PCR assays, further studies are needed to characterize the arenaviruses circulating in Finland.

No cowpox virus antibodies were detected in the house mouse from Suitia but one antibody

Table 1. Number of antibody positive animals tested with LCMV-, CPXV-, and PUUV- or DOBV-IFA. The Suitia research farm was trapped in 2004–2006, and the other sites* in 2001–2005.

Host species	Number tested	LCMV	PUUV/ DOBV	CPXV
Suitia				
Mus musculus	110	4 (3.6%)	0	0
Apodemus flavicollis	26	2 (7.7%)	0	0
Microtus agrestis	9	0	0	1 (11.1%)
Myodes glareolus	6	0	2 (33.3%)	0
Micromys minutus	2	0	0	0
Sorex araneus	6	0	0	0
Sorex minutus	1	0	0	0
Total	160	6 (3.8%)	2 (1.25%)	1 (0.6%)
Other sites*				
Mus musculus	7	0	0	1 (14.3%)

*Alavus (N = 1), Ulvila (N = 3), Uurainen (N = 2) and Parikkala (N = 1); LCMV = lymphocytic choriomeningitis virus, CPXV = cowpox virus, PUUV = Puumala virus, DOBV = Dobrava virus

positive house mouse was caught in Alavus. The occurrence of the cowpoxvirus is known to vary considerably both spatially and temporally (Kallio-Kokko *et al*. 2006, Laakkonen *et al*. 2006). The presence of cowpox antibodies in mice have been uncommon elsewhere (Crouch *et al*. 1995). Of hantaviruses, Puumala virus antibodies were found in two *Myodes glareolus* which is the natural host of this virus (Kallio-Kokko *et al*. 2005).

Fungi of the genus *Pneumocystis* have been reported from many rodent species in Finland, but previously not in *Mus musculus* (Laakkonen 1998). The prevalence of *Pneumocystis* in the house mouse revealed in this study (7.3%) was similar to those (range 6%–9%) found in this host species elsewhere in Europe (Laakkonen 1998). *Pneumocystis* organisms represent a large group of species of atypical fungi with cosmopolitan distribution and pulmonary tropism. Each species has a strong specificity for a given mammalian host species (Cushion 2004). The species occurring in wild house mice at least in the United States is named *Pneumocystis murina*, a species found also in laboratory mice (Keely *et al*. 2004). Of other fungi, *Emmonsia parvum* was found at a low (0.9%) prevalence in the present house mouse sample. This fungus species was suggested to be rare in rodent species that do not build their nests in the soil such as the house mouse (Hubálek 1999). In Finnish rodents, *Emmonsia parvum* is consistently found at low prevalence (< 10%) in many vole species (Soveri *et al*. 2000) but it is very rare in shrews (Laakkonen *et al*. 1999).

The house mouse is known to harbour several species of eimerian and other coccidian species in Europe and other continents (Levine & Ivens 1990). Coccidia, however, were not found in any of the house mouse individual under study. Several factors probably contributed to this result. A minority (12.7%) of the house mice caught were young individuals (< 10 g) in which eimerian species primarily occur. Furthermore, as stated in the introduction, invasive species often carry only a subset of their natural parasite fauna during colonization of a new area (Fromont *et al*. 2001). This could also partly explain the lack of parasites in the blood smears of mice studied. The scarcity of blood parasites in *Mus m. domesticus* has been reported previously from island populations (Moro *et al*. 2003). The blood smear technique we used in the present study may underestimate the blood parasite diversity due the difficulty of finding the smallest hemotrophic organisms in subtle infections. For example, the zoonotic bacteria *Bartonella grahamii* is known to occur in many rodent species including the house mouse (Ellis *et al*. 1999, Holmberg *et al*. 2003). Occurrence of hemoparasites also varies according to the season, prevalences being lowest during colder months (Wiger 1979).

The kidney forms of the blood parasite *Trypanosoma musculi* we found in house mice are host-specific parasites that live extracellularly in the blood. After immunoclearance of the flagellate form from the general circulation, mice are resistant to reinfection but parasites persist in the vasa recta of the kidneys. Mice acquire infective forms of the parasite from fleas of the

Table 2. Fleas found in small mammals trapped at the Suitia farm in 2004–2005.

Host species	Fleas species	Male	Female
Mus musculus	*Nosopsyllus fasciatus*	2	
	Ctenophthalmus agyrtes agyrtes	1	1
	Peromyscopsylla silvatica	1	
Apodemus flavicollis	*Nosopsyllus fasciatus*	3	1
	Ctenophthalmus agyrtes agyrtes	5	2
Microtus agrestis	*Ctenophthalmus uncinatus uncinatus*	1	3
	Megabothris walkeri	1	
Myodes glareolus	*Ctenophthalmus agyrtes agyrtes*	2	2
	Nosopsyllus fasciatus	0	1
Sorex araneus	*Doratopsylla d. dasycnema*	0	2
Sorex minutus	*Ctenophthalmus agyrtes agyrtes*	2	0

genera *Ctenophthalmus*, *Leptopsylla* and *Nosopsyllus* (Monroy & Dusanic 2000). There are no previous reports of *T. musculi* from wild house mice in Finland. Of other blood parasites, the pulmonary form of *Hepatozoon* found in *Myodes glareolus* is most likely *Hepatozoon erhardovae*, a species commonly found in this host species also in Finland (Smith 1996, Laakkonen *et al.* 2001).

Of the fleas found on house mice in this study, *Peromyscopsylla silvatica* has not previously been found on this host species in Finland (Smit 1969). *Peromyscopsylla bidentata* has a wide geographic distribution occurring on many rodent species (Smit 1969). *Nosophyllus fasciatus* is a very common flea of *Rattus* spp., and other rodents. *Ctenophthalmus agyrtes agyrtes* is often found on mice and voles throughout temperate regions. The number of fleas we found was low at least partly because collections were made from dead hosts. Ectoparasites tend to leave their host shortly postmortem once the core temperature drops to ambient levels.

Our results on the genetic variation of the house mouse partly supports the previous study (Prager *et al.* 1993) indicating that the Scandinavian mice are genetically a mixture of *musculus* nuclear and *domesticus* mitochondrial genomes. In addition, however, our finding of the mice from Ulvila in western Finland with the *musculus* mtDNA genome suggests a recent introgression from an area outside the regions investigated (*see* also Prager *et al.* 1993).

Although the possibility of a northern route of mice from Sweden to Finland cannot be excluded, it is probable that mice entered southern Finland from southeast Sweden via the chain of islands connecting these areas. Besides coming from the south through the present Denmark and southern Sweden, the other main recolonization route for the Finnish fauna during the last postglacial period was from the east through present southeastern Finland. Some species managed to use both colonization routes (Jaarola *et al.* 1999). Whatever the colonization route, the apparent multiple introductions indicate that the spectrum and diversity of pathogens carried by the house mice may vary considerably between different populations.

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