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Rapid field test for detection of hantavirus antibodies in rodents

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SUMMARY

Puumala virus (PUUV) is the causative agent of nephropathia epidemica, a mild form of haemorrhagic fever with renal syndrome. PUUV is transmitted to humans via aerosolized excreta of the infected bank vole (*Clethrionomys glareolus*). Current methods for screening of the PUUV prevalence among bank vole populations are laborious, combining sampling in the field and subsequent analyses in the laboratory. In order to facilitate animal testing, a new serological immunochromatographic rapid test was developed. The test uses PUUV nucleocapsid protein as antigen, and it detects anti-PUUV IgG antibodies in rodents. With fresh and undiluted bank-vole blood samples ($n=105$) the efficacy of the test was 100%, and with frozen and diluted samples ($n=78$) the efficacy was 91%. The test was also shown to detect related hantavirus infections in Norway lemmings and sibling voles ($n=31$) with 99% efficacy. The test provides an applicable tool for studying PUUV and related hantavirus infections in arvicoline rodents.

INTRODUCTION

Puumala virus (PUUV) belongs to the genus *Hantavirus* in the family Bunyaviridae [1] and is widespread throughout Europe [2, 3]. PUUV is the causative agent of nephropathia epidemica, a mild form of haemorrhagic fever with renal syndrome (HFRS). The bank vole (*Clethrionomys glareolus*) is the host species of PUUV, and the infection is transmitted to humans by aerosolized excreta from the chronically infected carrier rodent [4, 5]. PUUV is the most common hantavirus in Europe. Thousands of human cases are reported annually in northern Europe, the Baltic countries, Russia and central Europe. However, it is clear that most PUUV infections are subclinical or remain undiagnosed [6]. Human outbreaks of hantavirus infections can be predicted by population studies on the carrier rodents, because high numbers of

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carrier rodents correlate to the number of human infections [6–8].

The transmission dynamics of PUUV among bank vole populations depends on the density, behaviour and demographic factors of the population [7, 9–11]. The most probable ways for bank voles to acquire PUUV infection are behavioural contacts during mating, fights or communal nesting, and contact with excreta of other individuals [12]. Laboratory and experimental studies suggest that persistent hantavirus infection causes no clinical signs, and does not affect the normal behaviour of the rodent hosts [9, 13–15]. After exposure to a PUUV-contaminated environment, infectious virus persists in the tissues of the animal for at least 15 months [9], and probably for life. The voles continue to excrete infectious virus, even though an antibody response towards the virus develops. Puumala virus nucleocapsid protein (PUUV-N) has been shown to be highly immunogenic in animals, and specific antibodies appear in the blood of the vole approximately a week after the infection and persist for at least 15 months [9].

Current methods for screening for hantavirus antibodies in rodent populations are laborious, combining sampling in the field and subsequent analyses in the laboratory. Here we describe a new method for rapid detection of hantavirus IgG-class antibodies in mouse-like rodents that uses immunochromatographic test technology, described earlier by Hujakka et al. [16].

METHODS

The antibody test used purified baculovirus-expressed PUUV-N [17] as antigen, and the test result was detected using gold particles conjugated to rabbit anti-mouse IgG antibodies. The test was performed at room temperature by adding the blood sample (5 μl) and running buffer (90 μl) to the sample well. After 10 min the test result was read visually. If two visible red lines appeared (test and control line), the result was interpreted as positive. Only one visible red line (control line) meant that the test sample was negative but that the test had been performed correctly.

Minimum requirements to perform the rapid test include a flat and dry surface, a pipette, a temperature between +10 and +30 °C, and brief training for those performing the test. The test device can be stored for at least a year at almost any temperature but the running buffer needed for analysis should never be frozen.

A total of 204 blood samples from different bank voles and 31 serum samples from other arvicoline rodent species were used to evaluate the rapid test. The bank-vole blood samples were collected in central Finland in 2002. Wild bank voles were trapped with Ugglan Special live traps (Grahnab, Hillerstorp, Sweden). Samples were collected by cardiac puncture from anaesthetized (by carbon dioxide) bank voles, which were afterwards euthanized, or with an 18 μl capillary tube (Hemacrit tube, Hirschmann Laborgeräte, Eberstadt, Germany) from the retro-orbital sinus of live bank voles. Finally the samples were blown into cryogenic vials (Corning, Cambridge, MA, USA), from which the samples were transferred to the test device. When the samples partly clotted in the cryogenic vials, the remaining liquid phase was used for testing.

Panel I consisted of 105 bank-vole blood samples collected in October 2002. After trapping, the bank voles were transferred to the field laboratory, and sampled from the orbital sinus with the capillaries as described above. Immediately afterwards, the samples were assayed with the rapid test and then frozen for further use. The field laboratory was situated at room temperature near the trapping place. Panel II consisted of 21 bank-vole blood samples collected in August 2002. These samples were taken by cardiac puncture of anaesthetized animals, and frozen in 300 μl aliquots at −20 °C without any additives. The samples were assayed with the rapid test 9 weeks after the sampling at Department of Chemistry, University of Kuopio. Panel III consisted of 78 bank-vole blood samples collected in June 2002 from voles that were individually caged in an animal laboratory. These voles had been tested negative for PUUV antibodies with immunofluorescence assay (IFA) 3 weeks before the sampling for the rapid test. Animals tested as PUUV IgG-positive with IFA, were caged in the same room with the 78 negative animals for 2 days before they were separated. This enabled the horizontal transmission of PUUV among bank voles. The blood samples were taken with a capillary tube as described above, and stored in the capillaries for 2 days at +4 °C. After storage, the samples were transferred to cryogenic vials, and frozen in 5 μl aliquots at −20 °C without any additives. The samples were thawed and diluted 1:10 with phosphate-buffered saline (PBS) (pH 7·4) before assaying with the rapid test in laboratory conditions.

Panel IV consisted of 31 frozen serum samples, of which 26 were from Norway lemmings (*Lemmus*

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lemmus) [18] and five from sibling voles (*Microtus rossiaemeridionalis*) [19]. Of the lemming samples, 20 were from wild Norway lemmings, and 6 were from lemmings experimentally inoculated with Topografov hantavirus (TOPV) [18]. The five *Microtus* voles were experimentally inoculated with Tula hantavirus (TULV) [19]. The serum samples were frozen at −20 °C. All samples were assayed with the rapid test and with the IFA reference method.

The rapid test results were interpreted independently by two (panel I), five (panel II), six (panel III) or two (panel IV) people, who had no previous experience in immunochromatographic rapid tests and were briefly trained for reading the test. Only positive or negative interpretations were acceptable, and the readers were blinded from the IFA results. Assay performance parameters were calculated for each reader as correlations to the reference method. Efficacy was calculated from the formula: (specificity + sensitivity)/2.

An IFA [7, 20] was used as a reference method in this study. The method is based on PUUV-infected Vero E6 cells, which are acetone fixed on slide spots and stored at −70 °C until used for analysis. The rodent blood or serum was diluted 1:10 and 20 μl of the dilution was added to the slide spot. Specific antibody binding was detected using fluorescein isothiocyanate-conjugated anti-mouse IgG.

RESULTS

Of the 204 bank-vole blood samples, 54 were PUUV-N-specific IgG-positive and 150 were negative according to the IFA reference method. In panels I, II and III, there were 16, 7 and 31 PUUV-N-specific IgG-positive samples respectively. For panels I and II, the results were in total agreement between the rapid test readers and the reference method, showing 100% positive predictive value, negative predictive value, sensitivity, specificity, and diagnostic efficacy. Panel III was included in the study to compare the results of the test with fresh samples (panel I) and those stored. When the rapid test was used for samples of panel III, the mean value of the specificity was 96% and the sensitivity 87% (see Table). The specificity varied from 92 to 100%, and the sensitivity varied from 81 to 90% among the individual readers. Interpretations were discrepant for 17% (13/78) of the samples. The mean diagnostic efficacy was 91% ranging from 90 to 93%. The positive predictive value varied from 88 to 100% between individual readers, and the mean value was 93%. The mean value of negative predictive value was 92%, and it varied from 89 to 94% between individual readers.

Table. *Sensitivity, specificity and diagnostic efficacy of the rapid test with 204 blood samples and 31 serum samples, calculated as mean values for 2 (panel I), 5 (panel II), 6 (panel III), and 2 (panel IV) individual readers*

	Panel I	Panel II	Panel III	Panel IV
PUUV IgG-IFA				
Positive (*n*)	16	7	31	4
Negative (*n*)	89	14	47	27
Specificity	100%	100%	96%	98%
Sensitivity	100%	100%	87%	100%
Efficacy	100%	100%	91%	99%

Panel I included fresh blood samples, panel II frozen blood samples, panel III frozen and diluted blood samples, and panel IV frozen serum samples. IFA was used as a reference method.

Of the 26 lemming serum samples, three were found IgG-positive according to PUUV IgG-IFA reference method. Of the five vole samples, one was found IgG-positive with the reference method. One rapid test reader interpreted all the four IFA-positive and the 27 IFA-negative samples correctly. The other person interpreted the positive results correctly, but also one IFA-negative sample as positive.

DISCUSSION

Current methods for screening of hantavirus antibodies in rodent populations rely mainly on serology, in particular enzyme immunoassay (EIA) or IFA. Commercial products are not available for testing wildlife, and the present in-house laboratory methods adapt poorly to field studies due to their time-consuming and laborious procedures, and need for specific technical instrumentation. Additionally, hantaviruses require level-3 containment, and highly contagious animal samples pose a risk to the personnel involved in the analysis, sample handling and transportation. To minimize the biohazard risk it would be helpful not to transport these samples unnecessarily from the place of sampling. Thus, a rapid test performed at point-of-sampling has several advantages.

Pre-analytical factors (e.g. freezing or storage at +4 °C) clearly affected the quality of the samples in this study. The whole-blood samples assayed freshly (panel I) or after freezing in large volumes (panel II),

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gave 100% specificity as well as sensitivity for all the individual readers when compared to the reference method. However, blood samples from panel III, which were frozen as 5 μl aliquots and diluted 1:10 before assaying with the rapid test, gave slightly lower specificity and sensitivity than the samples in panels I and II. This decreased diagnostic performance may be partially due to too high pre-dilution or freezing of the blood in too small volumes (5 μl in 500 μl microtube). The antibodies may stay in the clot or suffer from concentrated enzymic activity due to evaporation or lyophilization of the sample. All the samples in panel III were collected from bank voles, which were negative when assayed with PUUV IgG-IFA 3 weeks before the sampling for the rapid test. Thus, the seropositive voles were in the early phase of seroconversion.

Furthermore, the reactivity of PUUV antigen with other hantavirus IgG-class antibodies was studied by analysing serum samples from Norway lemmings and sibling voles. Norway lemmings and sibling voles are the natural reservoir of TOPV and TULV respectively. TOPV nucleocapsid protein (TOPV-N) has a 13% difference in amino-acid sequence compared to PUUV-N [19], and between TULV-N and PUUV-N the difference is 21% [21]. Thus, serological reactions were expected between TOPV/TULV-specific antibodies and PUUV-N antigen. The rapid test found all of the four TOPV/TULV-specific IgG-positive serum samples, no false-negative results were detected, and only one false-positive result was interpreted by one of the two readers.

Although the PUUV rapid test shows adequate performance among closely related hantaviruses in the arvicoline host clade, it is possible that the PUUV-N antigen may not provide enough serological reactivity to hantavirus antibodies carried by sigmodontine (e.g. Sin Nombre) or murine [e.g. Hantaan virus (HTNV) and Dobrava virus (DOBV)] rodents. These rodents are phylogenetically more distant from arvicoline rodents, and the corresponding hantaviruses are also quite distant from PUUV [21]. Moreover, the reactivity between HTNV/DOBV-specific antibodies and the PUUV-N antigen has been shown to be low in a rapid test for human sera [22]. Studies to expand the rapid test technology to hantaviruses from the rodent subfamilies Sigmodontinae and Murinae are in progress.

The newly developed rapid test could be used for the screening of hantavirus antibody prevalence in rodent populations in field laboratories during longitudinal catch-and-release studies, as well as in laboratory animal houses. Overall, the rapid test had a good analytical performance when compared to the reference method, and met the requirement for a highly applicable field test. Blood samples can be taken from the retro-orbital sinus or the tail tip of the rodent with a small capillary, or using saphenous vein puncture, without harming the animals and enabling their release back to the wild, which is essential for long-term studies of transmission dynamics. Furthermore, the rapid test may provide an opportunity to predict outbreaks of nephropathia epidemica, and when extended to other emerging viruses, this test technology may provide a practical and rapid tool for monitoring and controlling other important zoonotic viruses.

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