Supplementary Table 1. Sequences of primers and probes and cycling conditions of quantitative and nested RT-PCR. Primers were used at the 10µM concentration.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Method | Primer and probe name and sequence (5’ to 3’) | Cycling steps | Temperature (°C) | Time | Number of cycles |
| Quantitative RT-PCR | Primer PUU R  (CCKGGACACAYCATCTGCCAT)  Primer PUU F  (GARRTGGACCCRGATGACGTTAA)  Probe PUU 1  (CAACAGACAGTGTCAGCA)  Probe PUU 2  (CAACARACAGTGTCAGCA) | Reverse transcription | 50 | 30 minutes | 1 |
| Hot Start activation | 95 | 10 minutes | 1 |
| Denaturation | 95 | 15 seconds | 45 |
| Annealing /Extension | 60 | 60 seconds | 45 |
| Cooling | 40 | 30 seconds | 1 |
| RT-PCR | Primer PPT334C  (TATGGIAATGTCCTTGATGT)  Primer PPT986R  (GCACAIGCAAAIACCCA) | Reverse transcription | 50 | 60 minutes | 1 |
| Denaturation | 94 | 2 minutes | 40 |
| Annealing | 44 | 60 seconds | 40 |
| Extension | 68 | 2 minutes | 40 |
| Final elongation | 68 | 7 minutes | 1 |
| Nested RT-PCR | Primer PPT376C  (CCIAGTGGICAIACAGC)  Primer PPT716R  (AAICCIATIACICCCAT) | Initial denaturation | 95 | 2 minutes | 1 |
| Denaturation | 95 | 30 seconds | 40 |
| Annealing | 44 | 60 seconds | 40 |
| Extension | 72 | 2 minutes | 40 |
| Final elongation | 72 | 10 minutes | 1 |

Supplementary Table 2. Detection of PUUV RNA in sera, lung, liver and urine of the 10 adult seropositive bank voles detected in natural populations. x : samples not available. N indicates that the qRT-PCR has been done but the cycle threshold was too high (above 45).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Region | Individual number | Sera (RNAcopies.µl-1) | Lung (RNA copies. mg-1) | Liver (RNA copies. mg-1) | Urine (RNA copies.µl-1) |
| Jura | NCHA00014 | N | 1.21e8 | 3.42e7 | N |
| NCHA00041 | N | 7.27e8 | 1.95e7 | N |
| NCHA00071 | 8.90e4 | 7.13e8 | 5.79e8 | N |
| NCHA00073 | 1.91e4 | 6.61e8 | 8.09e7 | x |
| NCHA00086 | 2.61e4 | 9.72e7 | 3.93e8 | x |
| NCHA000143 | N | 7.50e6 | 1.79e6 | x |
| NCHA000150 | 1.01e4 | 1.35e8 | 1.48e8 | x |
| NCHA000178 | 1.33e6 | 4.88e9 | 8.63e8 | 2.26e4 |
| NCHA000180 | 1.58e5 | 3.29e8 | 6.94e8 | x |
| NCHA000181 | 6.77e4 | 8.33e7 | 7.00e7 | 2.08e4 |

Supplementary Table 3.Anti-PUUV antibody responses of bank voles to PUUV infection through time. The amount of anti-PUUV IgG is expressed using the optical density measured at 450 nm (OD450nm). A sample is considered as seropositive when the OD450nm exceeds 0.1 (values are then indicated in bold). The three animals that died at 28 dpi are not included in the Table.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Region | Individual number | OD450nm | | | | | | | | |
|  |  | 0 dpi | 8 dpi | 14 dpi | 20 dpi | 28 dpi | 36 dpi | 43 dpi | 49 dpi | 55 dpi |
| Ain non-endemic | 15.129\_63 | 0.0000 | 0.0050 | 0.0700 | 0.0890 | **0.1270** | **0.2440** | **0.258** | **0.24** | **0.264** |
| 15.129\_67 | 0.0010 | 0.0025 | 0.0000 | **0.1090** | **0.1640** | **0.1510** | **0.155** | **0.144** | **0.118** |
| 15.129\_89 | 0.0000 | 0.0040 | 0.0380 | 0.0320 | 0.0750 | **0.1270** | **0.146** | **0.137** | **0.135** |
| 15.129\_92 | 0.0020 | 0.0210 | 0.0810 | **0.1430** | **0.1550** | **0.1650** | **0.217** | **0.172** | **0.196** |
| 15.129\_96 | 0.0030 | 0.0040 | 0.0050 | 0.0380 | 0.0700 | **0.1540** | **0.172** | **0.178** | **0.129** |
| 15.129\_98 | 0.0020 | 0.0675 | **0.1240** | **0.1910** | **0.1840** | **0.2050** | **0.173** | **0.154** | **0.172** |
| 15.129\_100 | 0.0030 | 0.0025 | 0.0090 | 0.0380 | 0.0850 | 0.0520 | 0.034 | 0.039 | 0.04 |
| 15.129\_66 | 0.0010 | 0.0310 | 0.0970 | 0.0420 | 0.0100 | 0.0150 | 0.018 | 0.028 | 0.042 |
| Jura  endemic | 15.131\_52 | 0.0000 | 0.0410 | 0.0850 | **0.1180** | **0.1120** | 0.0870 | 0.097 | 0.061 | 0.099 |
| 15.131\_53 | 0.0060 | 0.0615 | **0.1710** | **0.2450** | **0.2460** | **0.2950** | **0.441** | **0.405** | **0.27** |
| 15.131\_68 | 0.0050 | 0.0600 | **0.1020** | **0.1490** | **0.2550** | **0.3260** | **0.467** | **0.308** | **0.241** |
| 15.131\_70 | 0.0000 | 0.0310 | **0.1150** | **0.1330** | **0.2580** | **0.1890** | **0.285** | **0.25** | **0.206** |
| 15.131\_71 | 0.0070 | 0.0015 | **0.1310** | **0.1430** | **0.2550** | **0.2630** | **0.296** | **0.222** | **0.257** |
| 15.131\_72 | 0.0060 | 0.0925 | **0.1220** | **0.1830** | **0.1950** | **0.1820** | **0.235** | **0.213** | **0.198** |
| 15.131\_73 | 0.0000 | 0.0710 | 0.0950 | **0.1060** | **0.1680** | **0.1500** | **0.216** | **0.**098 | **0.143** |
| 15.131\_74 | 0.0000 | 0.0665 | **0.1220** | **0.1430** | **0.1640** | **0.1550** | **0.173** | **0.175** | **0.202** |

Supplementary Table 4. Detection of PUUV RNA in excreta collected at 8, 14 and 20 days post-infection using nested RT-PCR. - indicates bank voles that did not seroconvert during the experiment; + indicates bank voles that seroconverted during the experiment. POS indicates that the nested RT-PCR enabled to amplify PUUV RNA, whereas N indicates negative results.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Region | Individual number | Serological status | Sera | | | Feces | Saliva |
|  |  |  | 8dpi | 14dpi | 20dpi | 8dpi to 20dpi | 8dpi to 20dpi |
| Ain  non-  endemic | 15.129\_63 | **+** | POS | N | N | N | N |
| 15.129\_67 | **+** | N | N | N | N | N |
| 15.129\_89 | **+** | N | N | N | N | N |
| 15.129\_92 | **+** | N | N | N | N | N |
| 15.129\_96 | **+** | N | POS | POS | N | N |
| 15.129\_98 | **+** | N | N | N | N | N |
| 15.129\_100 | **-** | N | N | N | N | N |
| 15.129\_66 | **-** | POS | N | N | N | N |
| Jura endemic | 15.131\_52 | **+** | POS | N | N | N | N |
| 15.131\_53 | **+** | N | N | N | N | N |
| 15.131\_68 | **+** | POS | N | N | N | N |
| 15.131\_70 | **+** | N | N | N | N | N |
| 15.131\_71 | **+** | N | N | N | N | N |
| 15.131\_72 | **+** | POS | N | N | N | N |
| 15.131\_73 | **+** | POS | N | N | N | N |
| 15.131\_74 | **+** | N | N | N | N | N |

Supplementary Table 5.Detection of PUUV RNA in organs and excreta collected 55 days after experimental infections using quantitative RT-PCR. - indicates bank voles that did not seroconvert during the experiment; + indicates bank voles that seroconverted during the experiment. Viral RNA is expressed in RNA copies. mg-1. N indicates that the qRT-PCR has been done but the cycle threshold was too high (above 45).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Region | Individual number | Serological status | Sera | Lung | Liver | Kidney | Saliva | Urine | Feces |
| Ain  non-  endemic | 15.129\_63 | **+** | N | 4.07e7 | 3.07e6 | 1.76e6 | N | N | N |
| 15.129\_67 | **+** | N | 4.34e5 | N | N | N | N | N |
| 15.129\_89 | **+** | N | 5.03e6 | 1.11e6 | 1.48e6 | N | N | N |
| 15.129\_92 | **+** | N | N | N | N | N | N | N |
| 15.129\_96 | **+** | N | 3.32e6 | 1.69e6 | 1.71e7 | N | N | 1.64e7 |
| 15.129\_98 | **+** | N | 4.89e6 | N | 8.59e5 | N | N | N |
| 15.129\_100 | **-** | N | N | N | N | N | N | N |
| 15.129\_66 | **-** | N | 7.65e6 | N | 9.58e6 | N | N | N |
| Jura endemic | 15.131\_52 | **+** | N | 1.03e7 | N | N | N | N | N |
| 15.131\_53 | **+** | N | 6.35e6 | N | 2.00e6 | N | N | 3.55e6 |
| 15.131\_68 | **+** | N | 3.54e6 | N | 5.30e5 | N | N | N |
| 15.131\_70 | **+** | N | 4.30e6 | N | N | N | N | N |
| 15.131\_71 | **+** | N | 3.17e7 | 1.32e6 | 6.50e5 | N | N | N |
| 15.131\_72 | **+** | N | 8.90e5 | N | 5.19e5 | N | N | N |
| 15.131\_73 | **+** | N | N | N | 4.35e5 | N | N | N |
| 15.131\_74 | **+** | N | 5.74e5 | N | N | N | N | N |