

**Results:** High titre PVs were produced with titres between  $\sim 1 \times 10^8$  RLU/mL (*Ebolavirus/Cuevavirus*) and  $\sim 1 \times 10^{10}$  RLU/mL (*Marburgvirus*).

Lyophilised PV titres remained constant stored at  $-20^\circ\text{C}$  and  $4^\circ\text{C}$  for 12 months, while PVs kept at room temperature ( $22.5^\circ\text{C}$ ) demonstrated titre decreases of up to 3 orders of magnitude after 6 months. At  $37^\circ\text{C}$ , five log (*Marburgvirus*) or three log (*Ebolavirus* and *Cuevavirus*) decreases occurred after one month.

*Zaire Ebolavirus* (EBOV) antibodies showed no cross reactivity with native LLOV PVs. Furthermore, EBOV epitopes inserted into the LLOV GP and expressed on PVs had no significant impact on PV infectivity, and EBOV neutralising epitopes were successfully reconstituted in these chimeric antigens

**Conclusion:** In this study, high titre PVs were generated and found to be amenable to lyophilisation and long-term storage. Reconstituted PVs retained their function in neutralisation assays suggesting their structure is not compromised during freeze-drying. Insertion of epitopes in heterologous GPs did not impact infectivity or functionality. This data suggests a PV-based serological kit could be utilised in resource-limited countries for serological studies, after simple refrigeration storage.

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### Tula virus phylogeography

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**Purpose:** Tula hantavirus (TULV) is zoonotic virus widespread across Eurasia, where numerous small mammal species have been shown to be its reservoirs. In the Balkans, Serbia is the first country where TULV was detected, in European pine vole, *M. subterraneus* trapped in 1987. Although TULV is not considered pathogenic for humans, cases of human infection have been reported on several occasions so far.

Previously, we have shown the evidence of recombination events in studied TULV lineages from Serbia. In this study we applied Bayesian phylogeography framework to reconstruct the spatial and temporal dynamics of TULV based on sequences isolated from different geographical areas.

**Methods & Materials:** The analyzed dataset was made of 137 TULV S segment sequences existing in the database, including two sequences from Serbia; in total, 70 isolation sites within Europe and Asia, covering time span of isolation of 28 years (1987–2015) were included.

Sequences were aligned using CLUSTAL W implemented in MEGA 6 and then manually edited. The best fit nucleotide substitution model was determined by jModeltest 0.1.1 using all 88 proposed models. TreePuzzle was employed to investigate the phylogenetic signal of each sequence in the dataset. To explore temporal structure of the dataset, root-to-tip analysis was done by TempEst. The phylogeny, including phylogeographic distribution was co-estimated in a Bayesian framework using a Markov Chain Monte Carlo (MCMC) method implemented in the Beast package v 1.8.4

**Results:** Studied TULV strains formed three well supported clades matching the geographical origin: the clade closest to the tree root consisted of sequences from Russia and Kazakhstan; the second clade contained strains originating from western and central Europe; the third clade consisted of sequences from western and southeast Europe. The place of origin was assessed to be in *Kazakhstan*, with posterior probability of 1. The routes of viral spread included local distribution across *Kazakhstan* and Russia but also Europe, with the complex pattern of local viral migration further on.

**Conclusion:** The place of TULV origin was assessed to be in *Kazakhstan*, with westward spread leading to single introduction of TULV to Europe.

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### Serological study of leptospirosis in dogs from French Guiana

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**Purpose:** Leptospirosis is the most common zoonosis in the world and is considered as an emerging human disease. In French Guiana, recent epidemiological data indicate a significant increase in human cases since 2012, with an annual incidence in 2014 of 39 cases per 100,000 inhabitants, making it one of the major hotspots in the world. Considering this situation, the main goal of the present study was to investigate the incidence of the infection in dogs which are possible reservoirs or maintenance hosts for leptospires.

**Methods & Materials:** A serological survey was conducted in Guiana in 2016 on 95 dogs from Cayenne and Kourou. Location, race, sex, age, health and vaccination status were recorded for each dog. Sera obtained were tested for 27 serovars of pathogenic *Leptospira* species by the microscopic agglutination test. The results were interpreted according to the decision tree used in the VetAgro Sup leptospires laboratory, Lyon, France.

**Results:** Among the 95 samples, 59 showed agglutination at the cut-off point (1:20) for one or more pathogenic leptospiral serovars. Focusing on high titres ( $\geq 1:160$ ), seroprevalence was 11.6%. No statistically significant difference of prevalence due respectively to sex and age was observed ( $p > 0.05$ ). Icterohaemorrhagiae (40%), Australis (33.3%) and Canicola (27%) were the most frequently observed serogroups.

**Conclusion:** Dogs are not usually considered as a reservoir for *Leptospira*, except for Canicola, thus, the high prevalence found in this study in unvaccinated dogs probably results from a heavy exposure. However, the cut-off points selected and the absence of kinetic serology do not allow, in most cases, to conclude in favor of a current active infection. Dogs are highly exposed to pathogenic leptospires and humans living in the same environment are also at risk of infection. Thus, dogs could be considered as sentinels for human exposure to this zoonotic pathogen. In French Guiana, 98% of which is covered by equatorial rainforest, all the conditions are in place for the development of leptospirosis, particularly the climate which is characterized by abundant rainfalls and high temperatures

