Methods & Materials: A 77-year-old patient was hospitalized in the late summer of 2017 on the second day of the illness manifested by a fever up to 38.6 °C and diarrhea. The patient did not report recent travel but recalled mosquito bites. Past medical history included hypertension. At admission, routine laboratory tests, electrocardiogram (ECG) and chest x-ray were performed. In addition, cerebrospinal fluid (CSF), urine and blood samples were collected for a virological analysis.

Results: At admission, WBC count was 24.6 (reference range 3.4-9.7x10(9)/L) with neutrophilia (92%, range 44-72%) and very high levels of cardiac enzymes: creatinine phosphokinase 1856 (range 17-153 U/I), lactate dehydrogenase 433 (range 2-241 U/L), myoglobin 3116 (range 20-80 ug/L) and troponin I 17.640 (range 0.000-0.056 ug/L). ECG showed ST elevation. In the cardiac intensive care unit, an emergency coronary angiography was performed which confirmed the coronary artery stenosis. The patient's condition complicated on the 4th day of the illness by an altered level of consciousness with progression to coma, accompanied by neck stiffness and positive meningeal signs. Computed tomography of the brain was normal. Cerebrospinal fluid (CSF) showed pleocytosis with 26 cells/mm3, predominantly mononuclears (73%) and elevated protein level (1.151, range 0.170-0.370 g/L). Both CSF and urine were positive for WNV RNA by real-time and nested RT-PCR. Phylogenetic analysis showed WNV lineage 2. The patient was initially treated with acyclovir, ampicillin and cefepime parenterally with supportive therapy (antiedematous, antiaggregation and antihypertensive therapy). On the 8th day of the illness a respiratory insufficiency developed. The patient was intubated and mechanically ventilated, but developed hypotension and low oxygen saturation in spite of an adequate respiratory support. Despite the cardiopulmonary resuscitation, the patient died due to cardiopulmonary arrest.

Conclusion: Although cardiac involvement is not frequently reported in the course of a WNV infection, physicians should be aware of the possibility of a WNV-related myocardial infection.

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21.087

Isolation of *Brucella Melitensis* in Azerbaijan in 2014

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Purpose: Republican Anti-Plague Station (RAPS) in Azerbaijan provides confirmatory tests for especially dangerous pathogens (e.g.*Yersinia pestis, Bacillus anthracis, Brucella spp., Francisella tularensis*). In Azerbaijan RAPS and its regional Anti-Plague Divisions (APDs) are responsible for testing human samples for abovementioned pathogens. RAPS receives samples for confirmatory test from patients referred by Baku, regional hospitals and APDs.The aim of this study was to isolate and identify *Brucella* cultures from human blood samples.

Methods & Materials: In 2014, 1,137 blood samples were collected by RAPS from patients with clinical symptoms such as high temperature, perspiration, chills, myalgia, arthralgia, headache, fatigue, lack of appetite. All samples were tested using the Azerbaijan Ministry of Health (EDPs Laboratory Guidelines, 2013) algorithm via bacteriology and serology tests. Blood serum was tested for the presence of the antibodies of *Brucella spp*. via Huddleson reaction, Rose Bengal test and Wright's reaction. Samples positive for *Brucella spp.* per Huddleson and Rose-Bengal reactions further were tested by Wright's reaction. Positive blood samples by Wright's reaction with titers 1/200 and higher were then cultured. Isolated pure cultures were examined by biochemical (Urease, Oxidase, Catalase, TSI/H_2S, Dye sensitivities) and serology (Trypaflavine, Agglutination with specific polyvalent serum, anti -A and anti -M monospecific sera) tests.

Results: Data showed that 54.3% from total number of serum samples were positive by Huddleson reaction; 35.6% from total were positive by Rose-Bengal reaction and 38.7% from total samples were positive by Wright's reaction. Positive blood samples with titer 1/200 and higher from first diagnosed patients (n = 89) were cultured. All isolates were identified as *Brucella melitensis* (an overall isolation rate was 16.9%). The reports about positive results are sent to the Ministry of Health and State Veterinary Control Service (SVCS) and entered into the Electronic Integrated Disease Surveillance System (EIDSS) database of the Ministry of Health of Azerbaijan. Data from the system allows tracking the incidence of brucellosis and making operational decisions.

Conclusion: Future genetic analyses of these isolates can help identify subtypes of *Brucella melitensis* and establish the origin of *B.melitensis* circulating in the Azerbaijan territory and compare the results with those of neighboring countries.

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21.088

Generation, lyophilisation and epitope modification of high titre filovirus pseudotyped lentiviruses for use in antibody neutralisation assays

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Purpose: Filoviruses, such as *Ebolavirus*, are zoonotic pathogens causing disease outbreaks with high mortality rates, requiring scarce high containment facilities for research. Nevertheless, pseudotyped viruses (PV), consisting of a lentiviral core (plus luciferase reporter) and the envelope glycoprotein (GP), allow basic and translational virology to be conducted under low containment. Consequently, filovirus PVs were generated and viability assessed after lyophilisation and long-term storage. Next, antibody neutralisation tests were performed using native and hybrid GPs to assess differentiation between genera and species.

Methods & Materials: PVs were produced using a 3-plasmid transfection system (representing core, reporter and envelope) in HEK293T/17 cells, and supernatant titrated. Supernatants were then lyophilised in sucrose cryoprotectant solution, stored under various conditions, reconstituted and titrated. For antibody neutralisation tests, serially diluted, polyclonal convalescent sera (NIBSC, UK) or anti-GP monoclonal antibodies (Xiangguo Qiu, PHA, Canada; Erica Saphire, Scripps, USA) were incubated with PV for 1 h at 37 °C, prior to titration. To create artificial GP antigens, EBOV neutralising epitopes were inserted into the GP of another genus (*Cuevavirus*; LLOV) by mutagenesis, PVs generated and infectivity and neutralisation assessed.

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 °C and 4 °C for 12 months, while PVs kept at room temperature (22.5 °C) demonstrated titre decreases of up to 3 orders of magnitude after 6 months. At 37 °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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21.089

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.

Previousy, we have shown the evidence of recombinantion 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 Kazahstan; 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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21.090

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 (≥1:160), seroprevalence was 11.6%. No statistically significant difference of prevalence due respectively to sex and age was observed ($p > 0.05$). Icterohaemorhagiae (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