Results: All field serum samples tested turned serologically negative for PRVs. No cross-reactions have been detected between isolates and sera of PRV and MRVs employed in the study.

Conclusion: To the best of our knowledge Pteropine Orthoreoviruses have never been described to circulate in Europe and bats involved in their transmission cycle do not naturally occur in Europe. Indonesia/2010 is the ninth member of this viral species which has been described since the first description of these viruses in 1968, the fourth isolated from flying foxes, the first described in Europe although from healthy and legally flying foxes imported from Indonesia. In this study we decided to investigate whether PRVs had the chance to circulate within domestic and wild animals in Italy. We tested by a specific SN assay for PRV a total number of 769 serum samples from different domestic and wild animal species mostly coming from the Abruzzi region (central Italy). Moreover, 128 serum samples were from camels collected in Morocco during previous serological surveillance activities for other viral pathogens including West Nile and Bluetongue viruses. As expected, all samples turned out to be serologically negative for PRVs and no serological cross reactions were showed between PRV and MRVs used in this study. On the other hand, strong serological cross-reactivity has been previously described to exist within PRV isolates. This report describes a pilot study and certainly has some drawbacks. First, serum samples from domestic and wild animals were randomly selected and samples from bats and humans were lacking. Second, samples mainly originated from the Abruzzi region and from camels from Morocco. Therefore, the geographical origin of samples is not representative of the entire Italian territory. Indonesia/2010 reached Italy in 2010 with flying foxes legally imported from Indonesia. At the time of their arrival, the animals were straightly transported under sanitary conditions from the quarantine station of the Fiumicino airport (Rome) to a quarantine center in the municipality of Gatteo (Emilia Romagna Region). They remained in quarantine for 30 days before being shipped to an animal facility in Northern Europe. However, the chance of a spill-over event and infection of other animals or humans during their stay cannot be excluded. Moreover, PRVs may potentially already be present in Italy and Europe as for the chance of acquired infections during tourism in areas where flying foxes densely live. This event has been described indeed in a Japanese man who travelled to Bali in 2007. Epidemiological investigation by screening of 109 sera collected from human volunteers on the Pulau Island in Malaysia (where Pulau virus has been isolated from flying foxes) revealed that 14 of 109 (13%) were positive for Pulau virus, indicating that this group of viruses may be able to switch host and infect humans more frequently than other bat-borne viruses, such as the Nipah virus. Therefore, whereas human infection by PRV has been shown to occur after exposure to a flying fox, we do not have data supporting the infection in other animals. Overall, we investigated whether PRVs circulated within domestic animals in Italy and, based on the samples tested so far, there is no evidence of previous PRVs circulation. Certainly, more serum samples from different animal species and from different areas need to be tested and more importantly, human serum samples need to be included in the analysis. Pteropine Orthoreoviruses are able to cause respiratory tract infections in humans, therefore physicians should consider these pathogens in the diagnostic workflow particularly with patients coming from areas where flying foxes live or with patients who had contacts with them.

Application of silver nanoparticles to control Rift Valley fever virus infection in vitro and in vivo

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Objective: In this work we have tested the potential antiviral activity against Rift Valley fever virus (RVFV) of silver nanoparticles formulated as Argovit. Different formulations of silver nanoparticles have already been reported to display antiviral activity against several viruses belonging to different families. Compared to the classical antiviral strategies, the use of metal nanoparticles is a novel approach that poses many advantages, such as the non-emergence of resistant variants, as well as its safety and low cost. RVFV is a mosquito-borne pathogen causing an important disease in ruminants often transmitted to humans after epizootic outbreaks. Since there is no available treatment or licensed Rift Valley fever vaccine for human use, the development of new approaches able to inhibit viral replication and transmission for an efficient control of the disease is a must.

Methods: Argovit is a commercial formulation of silver nanoparticles provided by Vector-Vita Ltd (Russia) consisting of spheroid silver nanoparticles 35 nm-average-sized functionalized with polyvinylpyrrolidone (PVP) with

specific properties that increase silver nanoparticles stability and biocompatibility. The antiviral potential of Argovit against RVFV has been tested on Vero cell cultures and in an IFNAR (-/-) mouse infection model. In both systems, two different approaches have been assayed: (i) different dilutions of Argovit were added to previously infected cells or were administrated at different doses, routes and schedules to animals infected with a lethal dose of virus; (ii) virus was pre-incubated with different dilutions of Argovit before inoculation in mice or cultured Vero cells.
The effect of Argovit on viral infectivity was estimated by comparing virus production in cell cultures at the different Argovit conditions assayed; in the in vivo infection model clinical disease and death were compared between groups of mice receiving different treatments.

Results: In cells infected with RVFV, the presence of Argovit silver nanoparticles into the medium was able to control viral production in a limited manner, with a 50% reduction of the total virus yield. In contrast, preincubation of RVFV with Argovit concentrations near the citotoxicity threshold (0.2 mg/ml) abolished almost completely viral propagation, leading to a reduction of infectivity of 98%.

On the other hand, daily administration of Argovit by oral gavage to lethally infected mice, beginning one day after infection, slightly reduced the viral load in infected animals but this reduction was not enough to prevent their final death. In contrast, mice inoculated with a lethal dose of virus previously incubated with 20 mg/ml of Argovit silver nanoparticles showed a delayed-onset clinical disease and mortality, with a survival rate of 60%.

Conclusion: Although the ability of silver nanoparticles to control an ongoing RVFV infection in the conditions tested seems to be limited, the incubation of virus with Argovit before the infection leads to a clear reduction of its infectivity bath in vitro and in vivo. These results reveal the potential application of the microbicidal properties of silver nanoparticles to control the infectivity of this important zoonotic pathogen.

Application of viral metagenomics to investigate viruses circulating in wildlife-livestock interface in Mozambique

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Objective: Emerging infectious diseases (EIDs) are a major threat to human and veterinary public health. Majority of the viral EIDs originate from vector-borne viruses causing severe problems to the animal and human populations. Based on socio-economical, environmental and ecological factors different regions have been identified as so called hot spots where new EIDs are most likely to originate. One such hot spot is Mozambique, which is rich in arthropod vectors and wild life species: one of the predictors for originating new EIDs. In the study regions in Mozambique there is also a close proximity between wildlife, domestic animals and humans making the viral transmission from one species to another more likely to occur. Thus, it is important to identify and characterize the viruses circulating in these areas to prepare for future introductions of new pathogens and combat these infections. In this regard, the current aim of the study is to identify and see the prevalence of vector-borne viruses circulating in wildlife-livestock interface in Mozambique to be better prepared for upcoming emerging infectious diseases.

Methods: Viral metagenomics, broad-range PCRs and serological methods are used to investigate unknown and known viruses in Mozambique. Arthropod vectors (mosquitos and ticks) and serum samples from ruminants were collected from different farms in Mozambique during October/November 2014. Extraction of RNA from mosquitos and ticks was done after homogenization and DNAse treatment and these was then prepared for metagenomic analysis through high-throughput sequencing. The sequence data was analysed with bioinformatic tools for quality check, assembly and homology search to identify viral nucleic acids in the sample. Follow-up studies will be done with real-time PCR or conventional PCR to confirm the presence of viral nucleic acids in the sample and further characterize the identified viruses. In parallel, competitive ELISA has been performed to detect antibodies against Schmallenberg virus nucleoprotein in serum samples for cattle, sheep and goat. Nucleic acids has been extracted from the ruminants and arthropod vectors to be used in more specific broad-range PCRs.