## **RESEARCH ARTICLE**

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## Spatio-temporal trends and risk factors affecting West Nile virus and related flavivirus exposure in Spanish wild ruminants

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## Abstract

**Background:** During the last decade, the spread of many flaviviruses (Genus *Flavivirus*) has been reported, representing an emerging threat for both animal and human health. To further study utility of wild ruminant samples in West Nile virus (WNV) surveillance, we assessed spatio–temporal trends and factors associated with WNV and cross-reacting flaviviruses exposure, particularly Usutu virus (USUV) and Meaban virus (MBV), in wild ruminants in Spain. Serum samples from 4693 wild ruminants, including 3073 free-living red deer (*Cervus elaphus*), 201 fallow deer (*Dama dama*), 125 mouflon (*Ovis aries musimon*), 32 roe deer (*Capreolus capreolus*) and 1262 farmed red deer collected in 2003–2014, were screened for WNV and antigenically-related flavivirus antibodies using a blocking ELISA (bELISA). Positive samples were tested for neutralizing antibodies against WNV, USUV and MBV by virus micro-neutralization tests.

**Results:** Mean flavivirus seroprevalence according to bELISA was  $3.4 \pm 0.5$  % in red deer,  $1.0 \pm 1.4$  % in fallow deer,  $2.4 \pm 2.7$  % in mouflon and 0 % in roe deer. A multivariate logistic regression model revealed as main risk factors for seropositivity in red deer; year (2011), the specific south-coastal bioregion (bioregion 5) and presence of wetlands. Red deer had neutralizing antibodies against WNV, USUV and MBV.

**Conclusions:** The results indicate endemic circulation of WNV, USUV and MBV in Spanish red deer, even in areas without known flavivirus outbreaks. WNV antibodies detected in a free-living red deer yearling sampled in 2010, confirmed circulation this year. Co-circulation of WNV and USUV was detected in bioregions 3 and 5, and of WNV and MBV in bioregion 3. Sampling of hunted and farmed wild ruminants, specifically of red deer yearlings, could be a complementary way to national surveillance programs to monitor the activity of emerging flaviviruses.

Keywords: West Nile virus, Usutu virus, Meaban virus, Red deer Cervus elaphus, Risk factors, Wild ruminants, Spain

## Background

The distribution of vector-borne flaviviruses (family *Flavi viridae*) in the world has substantially increased over the last decades. During this period, many flavivirus infections have become a major public health concern due to continuous and growing reporting of outbreaks in humans [1]. Flaviviruses are mainly transmitted within an enzootic cycle involving ornithophilic mosquitoes or ticks as

competent vectors, as well as wild birds as the main amplifying hosts in the wild. Most mammalian species including humans are considered dead-end or incidental hosts, because they can get infected but are not thought to be able to transmit the viruses.

During the last few years, six flaviviruses, including West Nile virus (WNV), Usutu virus (USUV), tick-borne encephalitis virus (TBEV), Bagaza virus (BAGV), Meaban virus (MBV) and louping-ill virus (LIV), have been detected in Europe [2, 3]. Five of them have circulated in Spain in the last decade. WNV exposure has been documented in mosquitoes [4], wild birds [5, 6] and different mammalian



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