**Development of West Nile equine antibody ELISAs based of the E and NS1 viral recombinant proteins**

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**Objectives:** West Nile virus (WNV) is a vector-borne *Flavivirus* that is transmitted in an enzootic cycle between birds and by mosquitoes; incidental transmission to humans and horses occurs during periods of intense viral amplification (Castillo-Olivares *et al*., 2004). Mosquitoes of the genus *Culex* are the main vectors of WNV. A Previous study demonstrated that WNV circulates in Portugal at least since 2004 (Barros *et al*., 2011). In the last summer, 2 horse cases were identified and notified to OIE. The increase in diagnostic activity leads to the need for simple and specific serological methods that could be use in substitution of expensive commercial kits and virus neutralization test (VNT). The main goal of the present study was to establish specific diagnostic tests for WNV antibodies detection. We describe the preliminary results on the development of 2 indirect ELISAs that uses recombinant nonstructural NS1 and envelope (E) proteins as antigens for the detection of WNV antibodies in horses. The performance of the methods was compared with a commercial competitive ELISA and VNT.

**Material and Methods**

**Generation of recombinant E and NS1 proteins for development of indirect ELISAs:** In order to investigate the applicability of viral recombinant proteins in ELISA tests, the E and NS1 genes of WNV strain Eg101 (lineage1) (GenBank: AF260968) were cloned into vector Champion pET100 (Invitrogen), creating a His-tagged recombinant proteins, confirmed in the Western blot analysis by anti-His-tag monoclonal antibody as well as by WNV positive serum. The expressed proteins were used as antigen for developing indirect ELISAs. Recombinant proteins were solubilized from inclusion bodies as previously described (Barros et al, 2009).

**Indirect ELISA for detection of E and NS1 antibodies in horse sera:** A total of 1313 equine field sera, previously tested for antibodies to WNV by a commercial competitive ELISA (ID-VET, France) and VNT were used. Nunc maxisorb plates were coated with purified E or NS1 recombinant proteins by overnight incubation at 4°C. Wells were washed 3 times with PBS-Tween buffer (PBS, 0,05% Tween 20) and sera diluted (½) in PBS-milk buffer (3% E. coli lisate and 5% of milk), were added to wells. After incubation for 1 hr at 37°C, wells were washed as described above and incubated for 1h at 37°C with 100 µl of peroxidase-conjugated goat anti-horse antiserum (Serotec) diluted 1/5000 in PBS-milk buffer. After wash, each well received 100 µl of TMB solution. The color reaction was stopped after 20 min with 100 µl of 2.5 N sulfuric acid, and the plates were examined at 450 nm. A sample was considered positive if the optical density (OD) was greater than third the mean value of the negative controls. In order to established E and NS1 ELISAs, sensitivity and specificity of the two tests were evaluated using VNT as reference assay.

**Results:** Preliminary results showed that the correlation between in-house ELISAs and VNT for the detection of antibodies specific to WNV was strongly significant, so that sensitivity and specificity performances of these ELISAs, evaluated with respect to VNT, are very high.