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Development and evaluation of a new epitope-blocking ELISA for universal detection of antibodies to West Nile virus

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A B S T R A C T

West Nile virus (WNV) is an emerging zoonotic pathogen with a wide range of hosts, including birds, horses and humans. The development and evaluation of the performance of a new enzyme-linked immunosorbent assay (ELISA) are described for rapid detection of WNV-specific antibodies in samples originating from an extensive range of vertebrates susceptible to WNV infection. The assay uses a monoclonal antibody (MAb) which binds whole virus particles and neutralizes infection *in vitro* by recognizing a neutralizing epitope within the envelope (E) glycoprotein of the virus. This MAb, labelled with horseradish peroxidase, was used to compete with WNV-specific serum antibodies for virus-binding *in vitro*. The epitope-blocking ELISA was optimized in a manner that enabled its validation with a number of experimental and field sera, from a wide range of wild bird species, and susceptible mammals. The new ELISA exhibited high specificity (79.5–96.5%) and sensitivity (100%), using the virus-neutralization test as reference standard. It also required a much lower volume of sample (10 μl per analysis) compared to other ELISAs available commercially. This new method may be helpful for diagnosis and disease surveillance, particularly when testing samples from small birds, which are available in limited amounts.

1. Introduction

West Nile virus (WNV, family *Flaviviridae*, genus *Flavivirus*) is an arthropod-borne zoonotic, epizootic and epornitic pathogen. In recent years WNV has spread in many parts of the world, constituting a remarkable example of an emerging pathogen (Brault, 2009; Gould et al., 2001; Kramer et al., 2008). WNV is maintained in nature through a transmission cycle involving mosquitoes as vectors and wild birds as reservoir hosts. Epidemiologically, horses and humans are dead-end hosts since they acquire infection by infected mosquito bites and develop clinical illness, but are unable to transmit the virus. The most severe clinical outcome in these species includes neurological signs such as meningitis and encephalitis, leading to death. Case fatality in humans is approximately 3–4%, whereas in horses it ranges from 23 to 43%. Many bird species are susceptible to WNV infection, which can be fatal in some species (McLean et al., 2002). Individuals surviving the infection develop long-lasting immunity provided by specific antibodies in serum.

Serological tests for WNV-specific antibodies rely mainly on ELISA, hemagglutination inhibition or IFA techniques for screening, and virus-neutralization tests for confirmation (Shi and Wong, 2003). A critical issue regarding the specificity of these serological tests is cross-reactions with other flaviviruses. This is of particular importance in those areas where co-circulation of several flaviviruses occurs (Kuno, 2003). Among the above methods, virus-neutralization test provides the highest specificity, and for this reason it is considered as the "gold standard" for WNV-antibody detection. However, the virus-neutralization test is a complex, cumbersome and time-consuming technique, which is not suitable for large-scale testing in surveillance schemes. This method also requires live virus for testing samples, which involves the risks associated with manipulation of hazardous zoonotic pathogens. A high-containment facility (BSL-3) is required for sample processing, and is often not available. Of the above serological tests appropriate for the screening of antibodies to WNV in sera, ELISA is the format used most widely, as it is versatile, reproducible, and easy to standardize. Due to the clinical relevance of this disease

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