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Full Length Research Paper

A monoclonal antibody-based antigen-capture enzyme-linked immunosorbent assay (ELISA) for the detection of *bluetongue virus*

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A monoclonal antibody-based antigen-capture enzyme-linked immunosorbent assay (ELISA) was developed for the detection of *bluetongue virus (BTV)* in cell culture lysates and blood samples from sheep. The monoclonal antibody 3E2 and 1C11 specific to BTV VP7 were used as capture antibody and detection antibody, respectively. The assay has detected BTV 1-22 specifically, and had no cross-reactivity with the closely related epizootic hemorrhagic disease virus (EHDV) serotypes 5. The limit of sensitivity of the assay was 9 ng/ml for purified recombinant BTV VP7 and $10^{0.5}$ $TCID_{50}$/ml for BTV-5. The coefficient of variation (CV) of intra-assay and inter-assay range from 3.45 to 6.10%. The developed antigen-capture ELISA showed good coincident rate (100%) with INGEZIM BTV DAS in 5 serotypes BTV and 8 blood samples from sheep. Therefore, the antigen-capture ELISA may be useful for testing large number of samples in a convenient and short time.

Key words: *Bluetongue virus*, antigen-capture enzyme-linked immunosorbent assay (ELISA), serotypes, monoclonal antibody.

INTRODUCTION

Bluetongue (BT) is an insect-borne viral disease of ruminants. Among domestic animals, clinical disease occurs most often in sheep, and can result in significant morbidity. The economic consequences of the outbreak were dramatic. For instance, in the Netherlands, the estimated total net costs of the 2006 and 2007 outbreak were 200 million Euros (Velthuis et al., 2010). It has been included in the World Organization for Animal Health (OIE) list of notifiable diseases (formerly List A) (OIE, 2011). The distribution of BT is determined by the geographic distribution of the arthropod vector and extends globally between latitudes 35°S and 53°N (Martin et al., 2008; Orru et al., 2004). Outbreaks have occurred in many countries in northern and western Europe since 2006 (Saegerman et al., 2008; Carpentera et al., 2009; Kampen and Ortega et al, 2010).

Bluetongue virus (BTV) is the prototype member of the genus *Orbivirus* within the family *Reoviridae*. Thus far 26 serotypes are recognized. BTV is non-enveloped with a double shelled structure and a double-stranded (ds) 10 segment RNA genome. The virus contains 7 structural proteins and its genome encode also for 5 non structural proteins: NS1, NS2, NS3, NS3a and NS4. The outer capsid proteins, VP2 and VP5, are the serotype determinants and are responsible for generation of serotype-specific neutralizing antibody. The antibodies against VP7, the major core protein, will specifically detect the whole BTV serogroup. And the genomic segment 5, encoding NS1, is the most highly conserved of the 10 segments (Roy, 1989).

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