Abstracts

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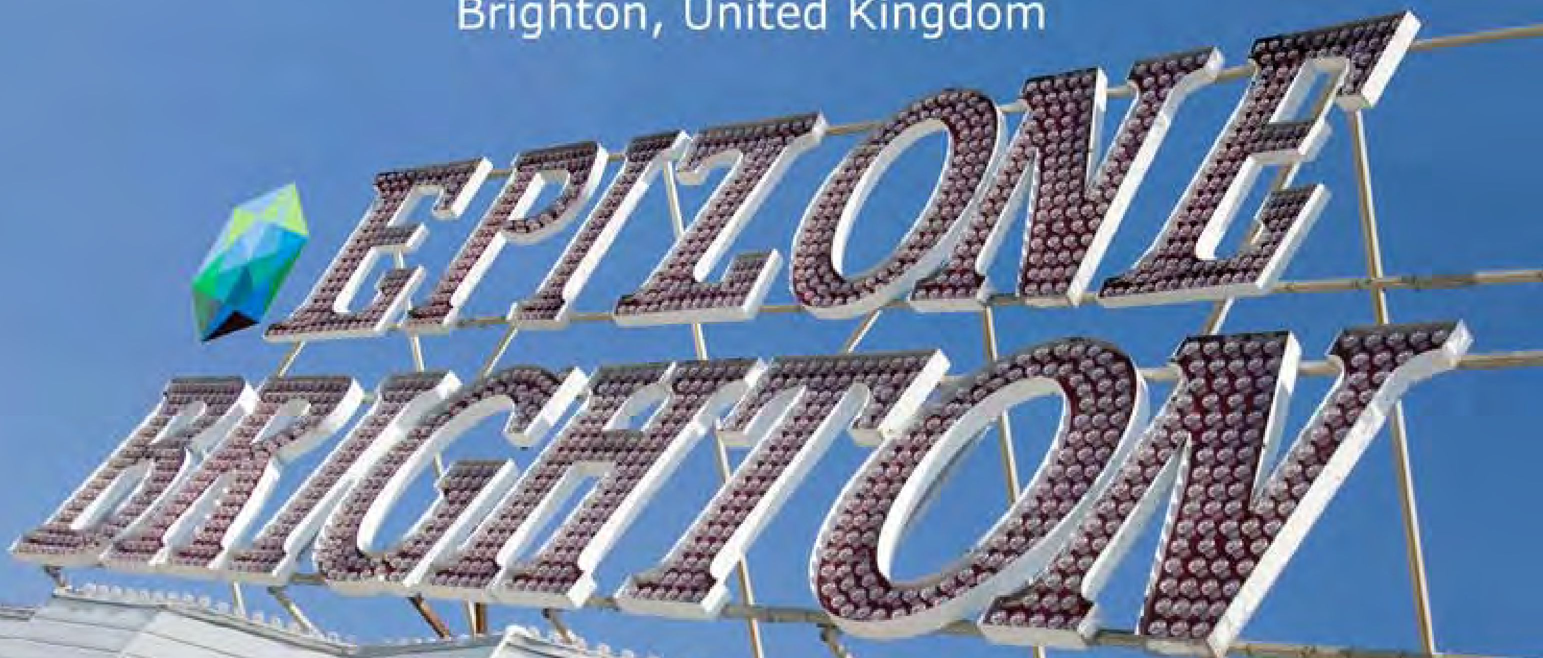

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D1: African horse sickness antibody detection by indirect immunofluorescence

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African Horse Sickness (AHS) is a vector-borne disease caused by an orbivirus of the family Reoviridae. AHS-Virus (AHSV) is transmitted by several Culicoides spp. The nine known serotypes circulate in Africa and serotype 4 and 9 have also circulated sporadically outside this continent.
All equidae can be infected. Four clinical forms are distinguished. Horsesickness fever, the mildest form, is mainly seen in vaccinated horses and in mules, donkeys or zebras. The most severe forms, the peracute (or pulmonary) and subacute edematous (or cardiac) ones are mainly observed in horses and lead to a morbidity of over 70%. A mixed form, where symptoms of both the pulmonary and cardiac forms are observed, is rarely clinically diagnosed.
The screening methods for antibody (Ab) detection are ELISAs (either indirect or competitive) homemade or commercial; and as confirmatory tests, immunoblotting and virus neutralisation (VNT) as well as complement fixation are described. Also indirect immunofluorescence (IIF) assays are published but the proposed protocols need highly skilled laboratory personal for accurate interpretation. Providing that a laboratory is able to work with cells, this method is technically easy to perform, inexpensive and fast. We therefore designed an improved, highly sensitive and easier to evaluate IIF, and compared it with a commercially available blocking ELISA (INEGZIM AHSV COMPAC PLUS).
In our assay, AHSV-9 infected Vero cells were mixed with the same amount of uninfected cells in each well of 96 or 24 well cell culture plates. The plates were fixed with aceton and stored either for few days at room temperature or for months at -20 °C.
Sera were incubated (diluted 1:10 in PBS, 45 min at 37 °C) and after a washing step, a secondary Ab (rabbit polyclonal Ab to horse IgG+IgM+IgA or IgG, 1:100 in PBS, 45 min at 37 °C) was added. After washing with PBS, the plates were rinsed with deionised water. Glycerol with 10% v/v of Tris-HCl, (0.01 mol/L, pH 8), was added to the wells to avoid drying and to achieve a brilliant staining. Plates were kept in dark at 4 °C until microscopic examination. The presence of typical intracellular inclusions bodies with a bright apple-green fluorescence indicated the presence of AHS-specific Ab in the tested sera. The evaluation was eased thank the mix of AHSV serotype 9-infected and uninfected Vero cells in the same well.
By analysing horse sera from AHS-vaccinated, experimentally infected, as well as non-infected and non-vaccinated animals, the relative diagnostic sensitivity and specificity of the IIF were 95.2% and 100% respectively, compared to the blocking ELISA. The IIF showed the same analytical sensitivity than the blocking ELISA. Although cells were infected only with AHSV-9, the test could detect antibodies against all the different AHSV serotypes. The method can be reliably used to detect Ab against all nine AHS serotypes, is highly specific and demonstrates a very high analytical and a good diagnostic sensitivity.
As the fixed plates are not infectious and can be stored without lost of quality at room temperature for several days, they can be shipped to laboratories that do not work with cells or AHSV.