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Assessment of 2 Commercial ELISAs for EIA and EVA Serology

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Introduction

Equine Infectious Anaemia

Equine infectious anaemia (EIA) is a disease with a world-wide distribution caused by a lentivirus. An outbreak of EIA started in Ireland in June 2006 and led to increased vigilance in the UK where the disease is notifiable. Once horses are infected they remain viraemic carriers for life, but also develop antibodies to the virus; these form the basis of routine screening tests. Traditionally an agarose gel immunodiffusion assay (AGID), is used for the detection of EIA (Coggins *et al* 1972) and this remains the prescribed test for international trade. However, this assay has an incubation period of up to 48 hours, is labour intensive, and requires experience in interpretation. Enzyme-linked immunosorbent assays (ELISA) for antibodies to EIA can be accurate and provide a quicker throughput of results, although it is still recommended that positive ELISA results are confirmed by AGID (USDA).

Equine Viral Arteritis

Equine viral arteritis (EVA) has a world-wide distribution and is caused by equine arteritis virus (EAV), an RNA virus classified in the family Arteriviridae. Occasionally seen in the UK, where it is notifiable, EAV infection can lead to a long-term carrier state in exposed stallions (Timoney and McCollum 1993) and are important epidemiologically; thus many horses are screened routinely for evidence of exposure prior to breeding. The virus neutralisation test (VNT) for the detection of antibodies to EAV, is considered to be the 'gold standard'. Although accurate, the VNT is slow and labour intensive and can be hampered by cytotoxicity in test samples. ELISAs which detect antibodies to EAV are available but have not been as extensively validated as the VNT; the prescribed test for international trade remains the VNT.

There is a need, particularly during the breeding season and when horses are moved internationally, for quick yet accurate tests for EIA and EVA. In both cases, a large population is screened and the requirement is for a test with high sensitivity, which will not fail to identify the small proportion of animals that are seropositive, yet will allow the majority of negative samples to be eliminated from more rigorous scrutiny. The aim of this investigation was to determine whether commercially available ELISAs could be of value when screening for each of these diseases in the diagnostic laboratory.

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Materials and Methods

EIA serology

A commercially available ELISA designed to detect antibodies to EIA virus (VMRD) was used to screen samples that had been screened previously using the AGID test (VMRD). Dilutions of the positive control samples from both kits were included to imitate positive test samples.

EVA serology

The 'Ingezim Arteritis ELISA' (Ingenasa) was used to screen 46 diagnostic samples for antibodies to EAV. These samples had been screened previously by VNT and samples that had shown cellular toxicity were included.

Results

EIA

All test samples were negative by AGID and ELISA. The positive control provided with the AGID kit was positive in the ELISA when used neat and when diluted to a concentration no longer detectable by AGID.

EVA

Nineteen negative and 27 positive samples, as determined by VNT were tested by the Ingezim ELISA (Figure 1). Eleven of the negative samples had shown some cytotoxicity when first tested by VNT and were pre-absorbed with cells before retesting. All of these 11 samples gave false positive results on the ELISA. Three of the eight non-toxic negative samples also gave positive results on the ELISA. Twenty-six of the 27 positive samples were positive by ELISA. The false negative sample had a titre of 4.5 $\log_2$ units for neutralising antibodies to EAV by VNT.

In this study, the ELISA had a specificity of 26% and sensitivity of 96%.

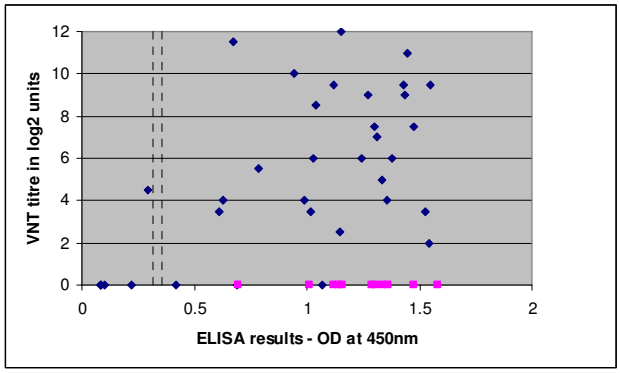

Figure 1. Graph of ELISA against VNT results for antibodies to EAV. Negative cytotoxic samples are pink. The hatched lines indicate the cut-off zone for the ELISA.

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Discussion

The EIA ELISA kit used appeared to give sensitive and reliable results and may be useful as a less labour intensive screening method than the Coggins test. Unfortunately, it could not be fully evaluated as no lack of positive test samples were available.

The ELISA kit designed to detect antibodies to EAV performed poorly in this study. Although differences might be expected between the VNT, which detects only neutralising antibody and the ELISA, which may detect antibodies to other viral components, this study suggests that this ELISA would be of little value routinely. The major drawback was the high false positive rate, which has been described previously (Cook *et al* 1989), and is associated with the presence of antibodies to tissue culture antigens in vaccinated horses. It also is of concern that a sample with a moderate antibody titre gave a negative result in the ELISA. Although exposure to infection might be expected to produce high antibody titres, missing an early rising titre could have significant consequences.

This simple investigation illustrates that although commercial assays for veterinary diagnostics are available, they may have inadequacies rendering them unsuitable for routine use. In-house validation is essential to ensure that kits perform satisfactorily and are appropriate for the intended purpose.

References

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