AASRP Research Summaries

Improved serodiagnosis of CAE and OPP: An investigation of unexpected positives caused by an interfering factor in fresh samples in isolated cases

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Introduction

Caprine arthritis-encephalitis virus (CAEV) and ovine progressive pneumonia virus (OPPV) are small ruminant lentiviruses (SRLVs) that persistently infect goats and sheep, causing significant economic loss for producers. An integrative program of serological testing to identify infected animals coupled with appropriate management practices is pivotal for disease control efforts. VMRD's SRLV cELISA kit is a USDA licensed assay widely used to detect antibodies to CAEV and OPPV in goats and sheep with excellent sensitivity and specificity. This study investigated reports of occasional anomalous positives in individual animals when samples were tested fresh that reverted to negative after the sample was stored. An improved version of the assay was optimized to accommodate for this sporadic issue with some freshly collected samples without sacrificing sensitivity or specificity.

Materials and Methods

A large set of serum samples were collected from a goat herd and tested within 6 hours (considered "fresh samples") using the original SRLV cELISA kit. Aliquots from samples that returned positive results were heat inactivated at 133°F (56°C) for 30 minutes, then run alongside the fresh samples to identify anomalous false positives. We then evaluated the samples in a new version of the SRLV cELISA. The improved SRLV cELISA was then validated using these samples as a part of a 269 field sample set. Sensitivity and specificity of the improved cELISA were compared to the original kit and a dot plot was generated to depict the distribution of positives and negatives relative to the cutoff of 30% I.

Results

The validation sample set of 269 revealed identical sensitivity and specificity of the improved SRLV cELISA as compared to the original version, with the only difference observed in fresh samples previously identified as false positive. Of the fresh sample set, 13 were identified as anomalous "false positives" in the original cELISA, with %I ranging from 35.6 to 50.7%. These fresh samples no longer ran positive in the improved kit; however, true positives continue to be positive. Heat inactivation of the false positive reactor samples at 133°F (56°C) for 30 minutes suggests potential interference by a heat-labile component in the problematic samples such as complement and/or clotting factors. Additional experiments to discern the exact nature of this interfering factor are underway.

Significance

The VMRD SRLV cELISA test has been a fundamental tool for the control of CAE and OPP for over a decade. It has a documented history of excellent performance, most recently demonstrated by its 100% accuracy in a ring trial performed by the Federal Research Institute for Animal Health in Germany. In most situations, samples are received by a diagnostic laboratory after being shipped or stored for a period of time. However, in isolated cases when samples are tested while fresh, it was found that an unidentified heat-labile factor could occasionally result in false positive results in the original assay. This was also consistent with previous anecdotal reports indicating that some animals with unexpected positive results had a recent history of vaccination or illness. If these anomalous positives were re-tested after storage, they would test negative, adding confusion to the scenario. The complicated circumstances surrounding false positive samples hampered initial identification and understanding of the issue. This targeted investigation enabled better characterization of the sample problem and optimization of the manufacturing process to address it. The improved SRLV cELISA resolves this false positive concern and accommodates for potentially problematic fresh samples, further enhancing test accuracy and CAE/OPP management efforts.