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Towards the veterinary diagnostics of the future

Main topic : Surveillance and control of emerging diseases

Development of a multiplex RT-qPCR to aid rapid equine infectious anemia virus detection and contribute to surveillance and management of equine infectious anemia disease programs.

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Introduction
The Equine Infectious Anemia Virus (EIAV) is the causal agent of the equine infectious anemia disease. This disease can cause significant economic losses in the equine industry worldwide. Today the control of EIA disease depends on the serological diagnosis, but the infected equids may not produce detectable anti-EIAV antibodies up to 157 days post-infection. This time when the horse is viremic, and we have no performing diagnostic tools, it is critical to control the disease spread. For several years, faster EIAV diagnosis techniques have been explored, and several RT-qPCR protocols for universal EIAV strain detection have been published. We have a large sample collection (>100) from spleen tissues of positive EIAV horses, and we have selected a panel of 42 samples, considering their diversity. We have tested this sample panel with up to 6 published protocols for RT-qPCR, qPCR, and PCR and one commercial kit for the detection of EIAV. The best results of detection for all of those tests only were able to detect 62% of the positive samples (1). It is for that a new approach to get an universal, and fast EIAV diagnostic tool is needed to improve the EIAV surveillance and the management programs in countries where the EIAV is endemic.
Material and methods
Recently our laboratory has developed a protocol to accelerate the molecular characterization of EIAV strains using targeted sequence enrichment and next-generation sequencing (2). We have processed more than 64 spleen samples from EIAV-infected horses today and taking advantage of the obtained sequence data. We have aligned those genetic data and identified potential conserved regions between different EIAV strains. Regarding those regions, several primers pairs and probes were designed.
Results
We have sequenced the completed genome of 14 EIAV strains (Only 15 completed EIAV genomes field isolated are published today in GeneBank) and sequenced more than the 80% of 50 EIAV additional EIAV strains in a few months. Using the EIAV sequences completed more than 80% and those accessible from GenBank, we did an alignment of 79 EIAV strains. This alignment presented a large diversity of EIAV strains and included a variety of outbreaks from around the world. The alignment result highlights 3 partially conserved regions where we could be able to perform PCR amplifications. Our RT-qPCR and PCR experiments showed successful amplification in those regions. The most performance pair of primers allowed us to identify 83 % of the infected horses from our test panel, an improvement of 20% over the best detection protocols available today. But, if we perform a multiplex RT-qPCR or qPCR using our best 2 pairs of primers, we increase our detection ratio up to 91% of the positive samples.
Conclusion
Our study is helping in the development of a universal PCR-based assay to early detect EIAV-infected horses by using innovative technologies of genome sequencing. These little steps will help in the surveillance and management programs for the EIA disease.