ISWAVLD 2023
International Symposium of the World Association of Veterinary Laboratory Diagnosticians
29 JUNE-1 JULY 2023
Congress Centre Lyon
Towards the veterinary diagnostics of the future

Main topic : Animal Health

Rapid detection of equine CXCL16 allelic variants associated with equine arteritis virus persistence

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Introduction

Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease of horses. Following natural infection, EAV can induce long-term persistent infection (LTPI) in the reproductive tract of stallions. Up to 70% of the infected stallions continue to shed EAV in semen for more than one year. Thus, LTPI stallions play a pivotal role in maintaining EAV in the equine population. Two alleles of the equine chemokine C-X-C motif chemokine ligand 16 ($CXCL16^S$ and $CXCL16^r$) were identified and correlated with the susceptibility or resistance of a subpopulation of CD3+ T cells to in vitro EAV infection and to the establishment of LTPI in stallions. Genotyping stallions based on $CXCL16^{S/r}$ would allow identification of those at the highest risk of establishing LTPI.

Methods

We developed a TaqMan® allelic discrimination assay for genotypification of equine CXCL16 gene targeting a single nucleotide polymorphism within exon 2. Blood samples from 160 horses spanning four different breeds were collected and genotyped using the new TaqMan® allelic discrimination assay. These results were compared to CD3+ T cell susceptibility to EAV infection by flow cytometry and DNA (Sanger) sequencing.

Results

Genotyping by Sanger sequencing determined that all horses with the resistant CD3+ T cell phenotype (n=56) were $CXCL16^{r/r}$ while horses with the susceptible CD3+ T cell phenotype (n=104) were either $CXCL16^{S/S}$ or $CXCL16^{S/r}$. Genotypification with the new TaqMan® allelic discrimination assay showed perfect agreement (100%) with Sanger sequencing and flow cytometric analysis. Genotype distribution by breed showed that Standardbred (92%) and Saddlebred (83%) horses most frequently carried the $CXCL16^S$ allele, followed by Quarter Horses (55%) and, lastly, Thoroughbreds, in which the $CXCL16^S$ allele was detected in approximately one-third (34%) of the population screened.

Conclusions

In conclusion, this new TaqMan® allelic discrimination genotyping assay provides a new diagnostic method for medium to high-throughput genotyping of equine CXCL16 with perfect agreement compared to Sanger sequencing allowing accurate identification of stallions at higher risk of becoming EAV carriers. Thus, it will assist with targeted vaccination practices and selective breeding with particular emphasis on stallions carrying the CXCL16S allele to help prevent the occurrence of the carrier state.