

the infection period. Since the virus undergoes rapid mutation following infection, the adaptive immune response must also evolve to meet this challenge. In order to fully understand the evolution of the T cell response during infection, we synthesized forty-four peptides, spanning the entire surface unit protein (gp90) of EIAV, and monitored the peptide-specific T cells responses both *in vivo* and *in vitro* over a six month period following challenge. One inapparent carrier (D64) and four recent EIAV infected ponies were included in this study. Peripheral blood mononuclear cells (PBMC) were isolated and stimulated separately with all 44 peptides. The EIAV gp90 epitopes-specific immune responses *in vitro* were determined by ELISPOT-IFN γ assay. In parallel, all peptides were injected intradermally and punch biopsies were collected for real-time PCR to monitor the cellular immune responses *in vivo*. As shown by both *in vivo* and *in vitro* assays, two of the four recently infected ponies recognized gp90 peptides three weeks post challenge. Similar to the CMI response to HIV infection, peptide-specific T cell recognition patterns changed over time. While some peptides were recognized throughout the sampling period, other peptides were only recognized at the later time points. Also, the response to some specific peptides disappeared after 6 months post infection. By contrast, peptide recognition by the inapparent carrier (D64) was more stable. The mechanisms responsible for this change remain unclear, but this dynamic shift in the immunodominant epitopes hierarchy in the newly infected horses may be the result of mutations in specific epitopes leading to an escape from T cell recognition. In the inapparent carrier, persistent recognition focusing on the more conserved peptides results in a more effective T cell response where virus replication is tightly controlled. These results indicate that T cell responses evolve during the early stage of EIAV infection. This interaction between virus mutation and T cell evolution needs to be considered when designing vaccines.

What feedback after five years from the implementation of the Italian National Surveillance Programme (NSP) for Equine Infectious Anemia (EIA)

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Introduction: since 2007, the Italian NSP for EIA imposes the annual sero-testing of the equine population, older than 6 months. Aims of the NSP are to: i. identify EIA outbreaks; ii. control infection spread through adoption of biosecurity measures; iii. evaluate EIA spatial and temporal trends; iv. identify infection clusters; v. provide evidence for further interventions/regulations. The objective of this study was to evaluate the trends of EIA prevalence in Italy since its adoption and estimate the risk for EIA infection for each equid species. **Materials and Methods:** the NSP data were analysed to estimate annual EIA seroprevalence by

Region and equid species. Seroprevalence trends were evaluated using the Chi-Squared test for linear trends in proportion, (LC 95%; $p < 0.05$). Odds Ratios were calculated to estimate the risk for EIA infection for each equid species. **Case definition:** equid testing ELISA or AGID-positive, confirmed in AGID or Immunoblotting. **Outbreak definition:** holding with at least one confirmed case. **Results and Discussion:** on average, about 228,000 equids were tested annually for EIA; 10,500 donkeys, 2,350 mules and 215,500 horses. Positivity in donkeys was occasionally detected while, a significant seroprevalence decrease was registered in horses: from 0.21% to 0.07%. Although the seroprevalence in mules was significantly higher than in horses, the decreasing trend was throughout the period, passing from 10.3% to 1.8%. However, from the data, the mule resulted to be about 50 times more at risk of resulting EIA positive compared to the horse (e.g. 2010: OR=51.4; IC95% 41- 64.4). Outbreaks dropped from 235 to 96 and in 2011, 80% were found to be incident and/or originating from previously not tested equids. **Conclusions:** clusters of infection were identified in Central Italy where EIA is endemic in draught animals, making them at a higher risk of infection while, EIA is sporadic in Northern Italy and Sardinia. In Southern Italy, while EIA is also considered as sporadic, a large population of equids has yet to be enrolled in the NSP. In general, the sport and racing horses are free from infection. The low incidence occurring in different Regions justifies the reduced frequency of testing introduced in the NSP in 2011. The control measures included in the surveillance programme were effective in lowering the seroprevalence and also the number of outbreaks especially in Central Italy. Nevertheless, focus must be put on the equine population living in rural conditions as well as horses not yet enrolled in the NSP, since they still might represent a risk for EIA spread. An additional risk, not to be underestimated, is the low sensitivity of the still recommended test, which is the agar gel immunodiffusion.

Genomic and Immunologic analysis of the Chinese attenuated EIAV vaccine

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The development of lentiviral vaccines is extremely difficult due to the broad genomic variation and fast *in vivo* evolution of the viruses. An attenuated vaccine against equine infectious anemia virus (EIAV) was developed by Shen et al in China in 1970's by serially passing a pathogenic wild-type strain in donkeys for 110 generations followed by 121 *in vitro* passages in donkey monocyte-derived macrophages. Laboratory experiments and country-wide vaccination confirmed that this attenuated EIAV vaccine effectively protected vaccinates from infection of pathogenic strains. To understand the mechanism that the EIAV vaccine effectively protects the infection of pathogenic strains, the genomic and immunologic characteristics of this vaccine were analyzed. Sequencing data of viral genomes revealed that the attenuating process of the