7 Summary

EHV-2 induced equine keratoconjunctivitis – Evaluation of the role of immunological mechanisms as well as of viral and bacterial co-factors

Several times, an involvement of the Equine Herpesvirus Type 2 (EHV-2) in different forms of inflammatory diseases of the cornea and/or conjunctiva in horses is documented; the exact pathogenesis of equine keratoconjunctivitis has yet not been understood completely. This project focus on the question, whether EHV-2-infected eye-diseased horses possibly show an immune status which differs from the reference value. Besides, the results of this thesis should provide conclusions, if the pathogenesis of the disease is possibly been influenced by immunosuppressive mechanisms of EHV-2. Furthermore, it should be investigated, whether other viral and/or bacterial pathogens play a role in equine keratoconjunctivitis. Finally, the investigations of the ocular tissue- and cell-tropism of EHV-2/-5 should further contribute to the understanding of the pathogenesis of this disease.

Investigations of the cellular immune status had been carried out comparing ten eye-diseased and 21 clinically sound horses. PBL, isolated from blood samples and swabs from the conjunctival fossa had been investigated using a EHV-2 specific nested PCR. Additionally, serological investigations had been carried out and the cellular immune status of each horse had been determined using flow cytometry and white blood cell count methods.

EHV-2 was detected within this study in both, eye-diseased and clinically sound horses by serological methods respectively nPCR. However, a statistically significant correlation between EHV-2 and the characteristic “eye-diseased” was not observed. Referring to the cellular immune status EHV-2 positive horses without ocular diseases showed significantly more often decreased b-cell numbers than EHV-2 negative horses with and without ocular diseases, respectively. This should be seen as first indication that an EHV-2 infection or reactivation causes a result in a measurable reduction of b-cells. Due to the small sample number, only further investigations will give information about, how individual differences of the immune system may influence the outbreak of keratoconjunctivitis induced by EHV-2.

To answer the question, whether other co-factors such as viral or bacterial pathogens are involved in EHV-2 induced equine keratoconjunctivitis, eye-swabs and blood samples from a second investigation group (consisting of 68 eye-diseased and 32 clinically sound horses) had been tested for EHV-2 and subsequently EHV-5, using specific nPCRs. Finally, random samples of eye-swabs had been investigated using pathogen specific PCRs for the presence of EAdV-1, chlamydiae and mycoplasmae, respectively. Within the scope of this project, the EAdV-1 specific nPCR had been established previously.

A statistically significant correlation between eye-diseased and sound horses relating to the detection rate of EHV-2 in swab- and blood-samples was also not observed in this investigation group. In addition, evidence for an involvement of EAdV-1, chlamydiae and mycoplasmae, respectively in inflammatory diseases of the cornea and/or conjunctiva of horses could not been deduced. However, it is very likely that EHV-5 is involved in the pathogenesis of equine keratoconjunctivitis as EHV-5-genom was detected frequently in diseased eyes compared with EHV-2, even though there was no observation of a significant correlation between the characteristic “eye-diseased”.

Studies about the tissue- and cell-tropism had been carried out on various ocular tissues (conjunctiva, cornea, nervus opticus and retina) as well as on cytobrush-swabs, which had all been taken *post mortem* from 14 healthy slaughtered horses. The genome of EHV-2 and -5 had been detected in cytobrush samples and in the conjunctiva only, even though the detection rate was very low (proportion of EHV-2 resp. -5 positive swabs: 11% in each case; proportion of EHV-2 resp. -5 positive conjunctiva: 7 resp. 8%).

Finally, the exemplary investigation of a EHV-2-nPCR positive conjunctiva of one horse using the *in-situ* hybridisation, a method that had been established within the scope of this project revealed, that EHV-2 DNA is present in the submucosa of the conjunctiva palpebralis. Thus, specific cells of the immune system might possibly be a site of latency within this tissue.