7 Summary

Survey of the antigenic protein fractions of a Sarcoptes mite extract by SDS-PAGE, Two dimensional electrophoresis and sequenz analysis

This research project was aimed to establish wich fractions of the complex protein solution of the Sarcoptes mite extract would be able to bind specific Sarcoptes antibodies and would thus be effective in an antigenic fashion.

For the succes of this experimental study a qualitatively and quantitatively sufiicient antigen preparation of mite extrakt solution was indispensible.

To achieve this objective, an extract of Sarcoptes scabiei var. vulpes was prepared by harvesting mites collected from the crusts of carcasses of naturally infested red foxes. The mites were subsequently isolated by a migration technique and then processed.

The protein mixture was produced through homogenization of the entire mitebody- also defined as „raw antigen“.

By applying the one dimensional SDS-PAGE the protein mixture could be separated into 33 bands in the range of 15-225 kDa.

18 of these protein bands were classified and bound by specific Sarcoptes antibodies in the immunoblot.

By using the technique of Two-dimensional electrophoresis, the proteins were arranged according to their distinct isoelectric points as well as their variable molecular weights. Thus it was possible to present the specific antibody binding protein bands in greater detail. Additionally, by applying this method the greatest biochemical purity of the protein spots could be achieved. This made the posterior sequenz of operation feasible.

Before undertaking the sequenz analysis the protein spots were blotted on a PVDF membrane and differentiated by Coomassie coloration. In the applied sequencing process 2 out of 8 spots yielded results.

The spots showed the following sequences:

Spot 11: (Ala/Ser) Pro Asn His Asp Lys Ala Phe Asp (Val/Glu) Leu – Val

Spot 12 : (Met/Gln) (Lys/Ser/Gly) Asn (Lys/Ser) Ala Val Leu Leu Gly Val Tyr Glu Asn – Asp