7 Summary

Molecular biological investigations for detection, epidemiological studies and differentiation of avian poxviruses

The aim of the present study was to establish a polymerase chain reaction (PCR) as molecular biological tool for routine diagnosis and differentiation of avian poxviruses. For testing the specificity of this PCR, fowlpox vaccine (FWPV HP-B) and samples from which fowlpox viruses were diagosed by conventional methods, were investigated. FWPV specific DNA was detected by amplifying a 578 bp fragment within the FWPV 4 b core protein gene. Examination of other avian viruses in this PCR revealed negative results. The agarose gel electrophoresis of PCR products was used for ascertaining the sensitivity of the PCR. FWPV specific DNA could be detected from a minimal amount of 150 copies of the genome. In comparison, the sensitivity was also ascertained by dot blot hybridization. With this technique a minimal amount of 75 copies of the genome could be detected. The sensitivity of the established PCR combined with agarose gel electrophoresis of PCR products seems to be sufficient and, because of its simple and rapid application superior to routine diagnosis. The established PCR was used to examine all samples, submitted for fowlpox virus diagnosis to the Institute of Poultry Diseases of the Free University Berlin between 2001 to 2003. After a distinctive increase of FWPV-outbreaks at the beginning of the investigations, a decrease was observed in 2002. Since the vaccination against fowlpox was not routinely used in the past, and after observation of several outbreaks till 2001, intensive vaccinations of poultry flocks were applied and resulted in distinct decrease in the numbers of cases in 2002. Futher, PCR in combination with restriction enzyme analysis (REA) was used as a molecular biological tool for differentiation of various avian poxviruses. With one primer set, it was possible to detect the DNA of 53 avian poxvirus strains or isolates from twelve bird species out of eight orders. REA of PCR products using *Eco*RV and *Nla*III allowed to differentiate these eight orders into six different restriction patterns in most cases. All investigated strains and isolates of fowl and turkey (order *phasianiformes*), pigeon and ostrich (orders *columbiformes* and *struthioniformes*), falcon (order *falconiformes*), and agapornis (order *psittaciformes*) had specific restriction patterns and were distinguishable from each other. Problems only occured with poxvirus isolates from birds of the order of *passeriformes*.

Isolates of a carrion crow, a pine grosbeak, three canary-birds and two sparrows had an identical restriction pattern („passeriformes-pattern“) but there were two exceptions: the canarypox virus strains KP-1-V557 and KP-1 as well as one isolate from a canary-bird showed a different restriction pattern, because of one additional *Eco*RV site in the PCR fragment, and the investigation of two isolates of sparrows had an identical pattern to strains and isolates of the order *phasianiformes*. The infections of sparrows by FWPV will be discussed in this work. In addition to the problem mentioned above, the isolates of a stone curlew (order *charadriformes*) and of a hawk (order *accipitriformes*) also showed the „passeriformes-pattern“. Nucleotide sequence analysis and phylogenetic analysis of amplified fragments of ten isolates and two strains confirmed the results obtained by REA.