7 Summary

A contribution to the occurrence of Mycoplasmas in birds of prey with conventional and molecular-biological methods

Mycoplasmas are pathogens of domestic poultry, causing important economic losses. Little is known about the occurrence and distribution of mycoplasmas in healthy birds of prey. Due to their fastidious culture requirements, their slow growth and the risk of contaminations with bacteria and fungi it is difficult to isolate mycoplasmas. In the present study PCR methods for the detection of mycoplasmas in birds of prey were established. In a second step the prevalence of mycoplasmas in these animals was investigated.

For the developement of a mycoplasma- multi- species PCR and species- specific PCRs for the detection of *M. gallisepticum* (MG), *M. synoviae* (MS), *M. iowae* (MI), *M. meleagridis* (MM), *M. buteonis*, *M. corogypsi*, *M. falconis* and *M. gypis* the 16S rRNS sequences of all 24 known avian mycoplasmas were compared.

A PCR developed by VAN KUPPEVELD et al. (1993) was modified for the mycoplasma-multi- species PCR. For the detection of MS and MM PCR methods discribed in the literature were evaluated. To detect *M. iowae*, MG/*M. imitans* as well as the mycoplasma species described by birds of prey (*M. buteonis*, *M. falconis*, *M. corogypsi* and *M. gypis)* new PCR methods were established. The PCR developed for the detection of MG/*M. imitans* focused on the possibility to differentiate of both species, as they can both occur in field samples.

The specifity of all PCR methods was confirmed using 23 avian mycoplasmas, further mycoplasma species isolated from mammals and walled bacteria. The differentiation of MG and *M. imitans* was possible throug Restriction Fragment Analysis using the enzymes ASEI and MSEI both generating different restrictionfragment patterns.

Using the PCR- protocols established in this study the sensitivity for the mycoplasma-multi-species PCR, the MS- PCR, the *M. falconis*- as well as the *M. gypis*- PCR was 1 pg DNA respectively 1 colony forming unit (CFU) per PCR reaction.

The sensitivity of the MG/*M. imitans*- PCR, MI- PCR and *M. corogypsi*- PCR was 100 fg DNA respectively 0,1 CFU, for the *M. buteonis*- PCR it was 50 fg DNA respectively 0,05 KbE per PCR reaction. The MM- PCR demonstrated a sensitivity of 10 pg respectively 10 CFU per PCR reaction.

In a following field study tracheal swaps of 60 healthy birds of prey, originating from captive and free ranging birds of prey were investigated using the the newly established PCR methods described above. Simultaneous tracheal swaps of these birds were used for

mycoplasma culture and followed by the identification of the isolates using an Immuno-Binding- Assay (IBA).

Mycoplasmas were isolated by culture in 76, 6 % (n=46) of the 60 tracheal swaps, 15% (n=9) were nagative and 8, 4% (n=5) were heavily contaminated by bacteria and fungi.

Using the mycoplasma-multi-species PCR, mycoplasma- DNA was detected in 88, 3% (n=53). A negative result was obtained in 8, 4% (n=5) and 3,3% (n=2) demonstrated an inhibition of the PCR.

Nestlings of Birds of prey demonstrated prevalence for mycoplasmas in 87, 5% by culture and 100% by PCR. Birds of prey found injured or bebilitated showed a prevalence for mycoplasmas in 88% by culture and 94,1% by PCR and captive birds a prevalence in 66,6% by culture and 77,8% by PCR.

54 isolates were recovered by culture and differentiated using IBA in *M. falconis* (n=12/20%), *M. gypis* (n=10/16, 7%) und *M. buteonis* (n=4/6, 7%).

With the species- specific PCR *M. falconis* (n=15/25%), *M. gypis* (n=11/18, 3%) und *M. buteonis* (n=11/18, 3%) were detected.

All mycoplasma species identified by IBA were confirmed by the species- specific PCR.

The MM-PCR according to BOYLE et al. (1995) revealed a positive result in eight samples (13, 3%). Confirmation of these results using a Restriction Fragment Analysis was negative and sequencing three representatives of these amplificates showed that they were identical but the similarity to the gen sequence of MM (L24106) was only 86%.

However, the length of the sequence examined is too small to state, that the detected organisms are a variation of *M. buteonis*. Further investigations are neededl to identify these mycoplasmas.

Pathogenic mycoplasmas like MG, MS, MI and *M. imitans* as well as *M. corogypsi* which has already been isolated from birds of prey were not detected in this study.

The results of this study demontrate that several mycoplasmas were not identified using the methods of this study. Twenty-eight (51, 9%) of the 54 Isolates could not identified by IBA and 23 samples (38, 3%) that were positive in the mycoplasma- multi- species PCR were not identified using the species- specific PCRs.

In those cases further investigations are also essential to identify these mycoplasmas.

The epidemiological study demontrated, that poultry pathogenic mycoplasmas seem to be rare in birds of prey. However, there is a high incidence of non-identified mycoplasmas.

Further it could be demonstrated that, using the species- specific PCRs in field samples of populations with unknown mycoplasma status, not only the specifity control of the method is

absolutly necessary, but also the establishment of further evaluation of the diagnoses with Restriction Fragment Analysis as well as sequencing is highly recommended.
The results of the MM- PCR according to BOYLE et al. (1995) showed that PCR methods, established for poultry, can not be used in other avian populations without further controls.