

## MYCOPLASMA AND AVIAN POLYOMA VIRUS INFECTION IN CAPTIVE SPANISH IMPERIAL EAGLES (*AQUILA ADALBERTI*)

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### Summary

Both avian polyomavirus (APV) and *mycoplasma spp.* can cause respiratory disease in birds. In birds of prey the detection of either of the two pathogens in relation to disease has been scarce until now. A Spanish Imperial Eagle (*Aquila adalberti*) that died despite treatment from severe anemia was found positive for *mycoplasma spp.* in all organs. Although cultures were negativ for cytopathogenic viruses, avian polyomavirus could be demonstrated by PCR (Polymerase Chain Reaction) in spleen and kidney. *Mycoplasma spp.* of yet unidentified species was cultured from 12 (67%) out of 18 captive Spanish Imperial Eagles examined subsequently, and APV could be isolated from two (11%) of the 18 healthy eagles. Mycoplasma or APV or both may have played a role in respiratory diseases such as Aspergillosis observed frequently in captive and free-living Spanish Imperial eagles in which analysis for these pathogens had not been performed. There is a need to better understand the role of these pathogens in captive and free living populations of eagles, especially the Spanish Imperial eagle.

### Zusammenfassung

Aviäre Mykoplasmen und aviäre Polyomaviren (APV) können schwere, häufig mit respiratorischen Symptomen verbundene Erkrankungen bei verschiedenen Vögeln hervorrufen. Bei Greifvögeln werden derartige Infektionen bisher nur selten diagnostiziert. Ein in Gefangenschaft gehaltener Spanischer Kaiseradler (*Aquila adalberti*) starb trotz Behandlung an einer schweren Anämie. Postmortal konnte aus allen Organen des Adlers hochgradig *Mycoplasma spp.* isoliert werden. Obwohl in der Zellkultur keine zytopathogenen Viren isoliert werden konnten, wurde mittels PCR (Polymerase-Ketten-Reaktion) in der Niere und der Milz des Adlers aviäres Polyomavirus nachgewiesen. Von 18 weiteren, in menschlicher Obhut befindlichen, klinisch gesunden Kaiseradlern wurden Rachen-, Tracheal- und Bindehautsacktüpfer untersucht. Dabei konnten bei 12 (67%) der Adler Mykoplasmen und bei zwei Adlern (11%) APV nachgewiesen werden. Die Spezies der isolierten Mykoplasmen konnte bisher nicht bestimmt werden. Sowohl Mykoplasmen als auch APV könnten eine Rolle bei den, bei freilebenden und in Gefangenschaft befindlichen Kaiseradlern häufig auftretenden Atemwegserkrankungen, meist Aspergillosen, spielen, bei denen bisher keine Untersuchung auf diese Erreger durchgeführt wurde. Weitere Untersuchungen zur Bedeutung von Mykoplasmen und APV für frei lebende und in menschlicher Obhut befindliche Kaiseradler wären von Interesse.

## Résumé

La mycoplasmosse et l'infection avec le polyomavirus aviaire sont des maladies graves souvent respiratoires dans les oiseaux. Au contraire ce ne sont pas de diagnostics fréquents chez les rapaces. Un aigle imperial espagnol (*Aquila adalberti*) qui présentait une anémie grave s'était mort malgré le traitement d'urgence. Les cultures de mycoplasmes étaient positives des toutes les organes de l'aigle. Utilisant des fibroblastes des embrions de poule il n'était pas possible d'isoler des virus cytopathogènes, mais avec le PCR (Polymease chain reaction) nous avons trouvé du APV dans le rein y la rate de l'aigle. Des échantillons de 18 aigles imperiaux ont été examinés en culture par mycoplasmes et dans des cultures des fibroblastes des embrions de poule. Dans 12 (67%) des oiseaux nous avons isolé du mycoplasme des espèces sans identifier, et dans les cultures du pharynx de deux aigles (11%) il y' avait APV. Dans les aigles imperiaux sauvages et on captivité il y a régulièrement des maladies respiratoires, fréquemment des aspergilloses. Les deux pathogènes isolés dans cette étude pourraient avoir un rôle dans ces épisodes, mais jusqu' à maintenant il n'y avait pas d'analyse de mycoplasme ou de APV. Il faut des études supplémentaires pour mieux entendre l'importance de ces deux pathogènes par l'aigle imperial sauvage et on captivité.

*Keywords: Birds of prey, Spanish Imperial Eagle, Avian Polyomavirus, Mycoplasma spp.*

## Extended Abstract

Avian polyomavirus virus (APV) infections recently have been demonstrated to affect birds of prey (2), such as the common buzzard (*Buteo buteo*) and the common kestrel (*Falco tinnunculus*). The virus was described originally as budgerigar fledgling disease virus and is known to cause severe fatal disease in psittacine and non psittacine birds worldwide (3, 6).

Mycoplasmosis is an important disease in poultry that is also known in birds of prey (1). Recent investigations have led to the detection of a number of species-specific new Mycoplasma species from birds of prey with respiratory disease (5). Demonstration of Mycoplasma infection on cytology or by other direct methods is difficult, as well as the isolation and identification of *Mycoplasma spp.* (4). As a result mycoplasmosis seems to be an underdiagnosed disease especially in free-living birds of prey admitted to rehabilitation centres (5).

This extended abstract describes a case of mycoplasmosis and APV infection in a captive Spanish Imperial eagle and summarizes briefly the results on the prevalence of Mycoplasma and APV in 18 captive Spanish imperial eagles.

An apparently healthy adult female Imperial eagle was found to be severely anaemic during a routine health survey. The bird was in very good body condition and showed very moderate respiratory distress. Despite the application of emergency treatment including homologous blood transfusions the eagle died within 24 hours. Macroscopic lesions observed during necropsy included the presence of numerous small (0.5 to 1 cm) granulomatous lesions in the fascia adjacent to the left shoulder joint, as well as in all air sacs (including the clavicular air sacs) and a very pale and atrophic spleen. Microscopically severe haemosiderosis was observed in the liver, in combination with a moderate diffuse infiltrate of mono- and polymorph-nuclear cells. In the lung few large pale eosinophilic intranuclear inclusions could be observed in the present macrophages

and some epithelial cells. Samples were processed for microbiology, isolation and PCR of *Mycoplasma spp.* as well as for virus isolation and PCR to detect avian polyomavirus DNA.

*Mycoplasma spp.* was cultured in large amounts from all organs, as well as from swabs from the mentioned shoulder joint. The isolate could not yet be identified, but using antisera against *M. synoviae* and *M. gallisepticum* it proved to be neither. Inoculated cultures of chicken embryo fibroblasts (CEF) were negative for cytopathogenic viruses. Using PCR, APV could be detected in the spleen and kidney of the bird. No pathogenic bacteria or fungi were found in the microbiological cultures.

Subsequently, tracheal, choanal and conjunctival swabs of 18 captive Spanish Imperial eagles were examined for the presence of *Mycoplasma spp.* by culture and PCR. In 12 (67%) of the analysed, clinically healthy birds *Mycoplasma spp.* were detected. In five birds *Mycoplasma spp.* could be grown from choanal as well as from tracheal swabs, while in five eagles only the choanal and in one bird only the tracheal swabs were positive. Only on one occasion *Mycoplasma spp.* could be cultured from a conjunctival swab. Pharyngeal and cloacal swabs of the same 18 eagles were examined for the presence of cytopathogenic viruses in CEF cultures. APV was isolated from pharyngeal swabs of two eagles (11%), while no cytopathogenic virus was found in the other samples. The two APV positive eagles had both presented respiratory symptoms upon admission at the rehabilitation and captive breeding centre a few month ago, but were clinically healthy at the time of sampling.

Both, *Mycoplasma spp.* and APV are known to cause respiratory disease in their hosts (1, 2, 3, 4). However, *Mycoplasma spp.* have also been isolated from wild injured or debilitated raptors that did not show signs of respiratory disease or affection of joints (4). A common kestrel from which APV could be isolated showed anaemia, ascites and fatty degeneration of the liver (2). In previous years, cases of Aspergillosis with severe pulmonary fibrosis have been observed in young, free-living Imperial eagles found debilitated or injured, in which viral cultures were negative but no PCR for APV or cultures for *Mycoplasma spp.* had been performed. Either one or both of the aforementioned pathogens may have played a role in these cases. Additional analysis are under way in order to better understand the importance, pathogenesis and epidemiology of APV and *Mycoplasma spp.* infections in captive and free-living populations of the Spanish Imperial eagle.

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