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OCCURRENCE OF MYCOPLASMAS IN FREE-RANGING BIRDS OF PREY IN GERMANY

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ABSTRACT: Mycoplasmas are well-known avian pathogens of poultry and some passerines. Although reported in birds of prey, their role as pathogens is still unclear. Healthy, free-ranging raptor nestlings sampled during a routine ringing (banding) program, and birds of prey from rehabilitation centers, tested positive for *Mycoplasma* spp. by culture and a genus-specific polymerase chain reaction (PCR). Given the lack of clinical signs and disease, we suggest that mycoplasmas in raptors may be commensal rather than pathogenic. Using immunobinding assay and species-specific PCR tests, *Mycoplasma buteonis*, *M. falconis*, and *M. gypis* were identified; *M. falconis* was only detected in falcons. Additionally, some isolates could not be identified. This is the first report of *Mycoplasma* spp. isolations from Western Marsh Harriers (*Circus aeroginosus*), a Eurasian Hobby (*Falco subbuteo*), and a Barn Owl (*Tyto alba*).

Key words: Accipitridae, molecular biology, *Mycoplasma*, *M. buteonis*, *M. falconis*, *M. gypis*, nestlings, PCR.

INTRODUCTION

Several mycoplasmas are recognized pathogens of domestic poultry, with which they cause significant economic losses. The potential for disease associated with mycoplasma infections in birds of prey is poorly understood, but respiratory dysfunction, air sacculitis, pneumonia, and tracheitis related to unspeciated mycoplasma infections have been described in raptors (Furr et al., 1977; Gylstorff and Grimm, 1987; Gerlach, 1994; Heidenreich, 1997). A novel mycoplasma that was described as *Mycoplasma corogypsi* was isolated from the footpad abscess of a Black Vulture (*Coragyps atratus*; Panangala et al., 1993), and *M. gallisepticum*, *M. gallinaceum*, *M. gallinarum*, and *M. iners* were isolated from two Peregrine Falcons (*Falco peregrinus*) with respiratory disease (Poveda et al., 1990). Three novel species, *M. buteonis*, *M. falconis*, and *M. gypis*, were isolated from Eurasian Buzzard (*Buteo buteo*), Saker Falcon (*Falco cherrug*), and Eurasian Griffon (*Gyps fulvus*), respectively (Poveda et al., 1994). These same *Mycoplasma* spp. were isolated from healthy raptors by Morishita et al. (1997). Additionally, the authors detected *M. anatis*, *M. columborale*, *M. gallisepticum*, *M. gallinaceum*, *M. gallinarum*, and *M. iners* in association with respiratory disease, and *M. corogypsi* from a case of pododermatitis. To date, *M. falconis* has only been isolated from falcons, but *M. gypis* and *M. buteonis* have been recovered from members of both the Falconidae and Accipitridae families (Lierz et al., 2000, 2002, 2008). Recently a new species, *M. vulturii* sp. nov., was isolated from an Indian White-backed Vulture (*Gyps bengalensis*; Oaks et al., 2004).

In a study of injured or debilitated birds of prey in Germany, *Mycoplasma* spp. were isolated from 82% of nestlings, 26% of juveniles, and 50% of adults (Lierz et al., 2000). Using an immunobinding assay (IBA), these species were identified as *M. gypis*, *M. meleagridis*, *M. falconis*, and *M. buteonis*; five isolates could not be identified. Although clinical signs were not observed in *Mycoplasma* spp.-positive birds, individuals with an air sac biopsy positive for *M. falconis*, *M. buteonis*, or *M. gypis* did exhibit a greater incidence of mild perivascular infiltration by lymphocytes or plasma cells of the air sac. A high prevalence of mycoplasma infection has also been reported from captive falcons;

100% of tested, healthy, captive falcons from a private collection in the United Arab Emirates were culture positive (Lierz et al., 2002). Using IBA, these isolates were identified as *M. meleagridis*, *M. corogypsi*, *M. falconis*, and *M. buteonis*; six isolates could not be identified. Positive results were also observed in captive birds of prey from Germany, where *Mycoplasma* spp. were detected by culture and by genus- and species-specific PCR methods in all tested falcons, Northern Goshawks (*Accipiter gentilis*), and a Eurasian Buzzard; however, all vultures were negative (Lierz et al., 2008). In that study, birds were infected with *M. falconis*, *M. buteonis*, *M. gypis*, and unidentifiable *Mycoplasma* spp. It is possible that the prevalence of mycoplasma in captive raptors is associated with their close contact. It is also possible that mycoplasmas may exist as a commensal organism of the tracheal mucosa of many raptors (Lierz et al., 2002).

In order to further evaluate the distribution of mycoplasma in raptors, it is necessary to examine healthy, free-ranging birds. To accomplish this, we tested adult raptors recently submitted to rehabilitation centers and nestlings sampled during a routine ringing program.

MATERIALS AND METHODS

Nestlings

Seven Northern Goshawks, seven Western Marsh Harriers (*Circus aeroginosus*), four Eurasian Buzzards, and five Red Kites (*Milvus milvus*), from nine different nest sites in Brandenburg, Germany, were examined for the presence of mycoplasmas using two tracheal swabs (one for culture and one for PCR), as described by Lierz et al. (2000). Sampling was performed during a routine ringing and health-screening program when the nestlings were between 30–50 day of age. Any bird showing any evidence of disease resulted in the entire clutch being removed from this study. All birds were placed back into their respective nests within 10 min.

Free-ranging birds of prey

Sixteen free-ranging birds of prey, found in the same area where nestlings were sampled, were also tested (Table 1).The birds originated from different rehabilitation centers and were only included in the study if they were found in otherwise good body condition after an accident and had not been resident in the center for more than two days.

Mycoplasma culture

Tracheal swabs were cultured immediately after sampling, using liquid and agar media as described (Bradbury, 1998), without thallium acetate. Swabs were immersed in mycoplasma broth and immediately subcultured onto solid mycoplasma agar, with both broth and agar incubated at 37 C in an atmosphere of 5% CO_2 for 5 days, followed by a second subculture onto solid media. Solid media were observed for the occurrence of mycoplasma colonies for up to 10 days using a light microscope. Isolated mycoplasma colonies were identified with an IBA according to Kotani and McGarrity (1985), using antiserum against the following avian reference strains: *M. anseris*, *M. buteonis*, *M. corogypsi*, *M. falconis*, *M. gallisepticum*, *M. gypis*, *M. iowae*, *M. lipofaciens*, *M. meleagridis*, and *M. synoviae*.

Polymerase chain reaction and restriction enzyme analysis

All tracheal swabs were first screened for the presence of *Mycoplasma* spp. DNA by using a genus-specific PCR as described by Lierz et al. (2007). Positive samples were further investigated using species-specific PCR tests for the detection of *M. buteonis*, *M. corogypsi*, *M. falconis*, and *M. gypis*; PCR products were subjected to a restriction enzyme analysis to ensure their specificity (Lierz et al., 2008).

RESULTS

Mycoplasmas in nestlings

Mycoplasmas were cultured from 21 of 23 (91%) tracheal swabs from nestlings (Table 2). Two swabs (9%) showed a heavy contamination by other bacteria. Using IBA, 11 isolates were identified as *M. gypis*; seven of those in a mixed infection with a second unidentifiable *Mycoplasma* sp. and, in one case, in a mixed infection with *M. buteonis*. In 10 cases, isolates of one bird could not be identified with the employed antisera.

By PCR, *Mycoplasma* spp.-specific

TABLE 1. Distribution of *Mycoplasma* spp. from free-ranging raptors, detected by culture and immunobinding assay (IBA), and by polymerase chain reaction (PCR).

Species	Differentiation by IBA	PCR-positive result[a]
Common Kestrel (*Falco tinnunculus*)	*M. falconis*	*M. falconis*
Common Kestrel	Contaminated sample	*M. buteonis*
Common Kestrel	*M. falconis*	*M. falconis* *M. buteonis*
Common Kestrel	*M. falconis*	*M. falconis* *M. buteonis*
Common Kestrel	*M. falconis*	*M. falconis*
Eurasian Hobby (*Falco subbuteo*)	Not identified[b]	*M. falconis*
Northern Goshawk (*Accipiter gentilis*)	Not identified	Negative[c]
Northern Goshawk	Not identified	Negative
Northern Goshawk	Not identified	Negative
Northern Goshawk	Not identified	Negative
Northern Goshawk	Not identified	Negative
Eurasian Buzzard (*Buteo buteo*)	*M. gypis*	*M. gypis* *M. buteonis*
Common Buzzard	*M. gypis*	*M. gypis*
Common Buzzard	*M. gypis* Not identified	*M. gypis*
Western Marsh Harrier (*Circus aeroginosus*)	*M. buteonis*	*M. buteonis*
Barn Owl (*Tyto alba*)	Not identified	Negative

[a] The *Mycoplasma* genus-specific PCR (Lierz et al., 2007) was positive in all of the birds; species-specific PCR tests for the detection of 16S rRNA gene of *M. buteonis*, *M. corogypsi*, *M. falconis*, and *M. gypis* were used (Lierz et al., 2008). Only the positive results are provided; the other PCR reactions were negative.

[b] Not identified with the IBA using antisera against *M. anseris*, *M. buteonis*, *M. corogypsi*, *M. falconis*, *M. gallisepticum*, *M. gypis*, *M. iowae*, *M. lipofaciens*, *M. meleagridis*, and *M. synoviae*.

[c] Negative in all *Mycoplamsa* spp.-specific PCR tests used.

DNA was detected from all 23 (100%) tracheal swabs (Table 2). The species-specific primers for *M. gypis* were able to amplify a product in 13 of 23 nestlings (57%). All five Red Kites, four of seven Western Marsh Harriers, two of four Eurasian Buzzards, and two of seven Northern Goshawks were positive using this PCR; the identity of all products was confirmed by restriction enzyme analysis. All 11 birds that were culture-positive for *M. gypis* tested positive by PCR. Only one Western Marsh Harrier nestling tested PCR-positive for *M. buteonis*, and all birds tested negative for *M. corogypsi* and *M. falconis*.

Mycoplasmas in free ranging birds of prey

Mycoplasmas were cultured from 15 of 16 (94%) tracheal swabs from free-ranging birds of prey (Table 1). One sample demonstrated a heavy bacterial contamination. Isolates were identified by IBA as *M. falconis* from four (25%) Common Kestrel (*Falco tinnunculus*); *M. gypis* from three (19%) Eurasian Buzzards; and *M. buteonis* from one (6%) Western Marsh Harrier. Eight isolates (50%) could not be identified, and in one case, a harrier was co-infected with *M. gypis* and *M. buteonis*.

Mycoplasma spp. DNA was detected in all birds using genus-specific PCR. *Mycoplasma* spp.-specific PCR tests were positive for *M. falconis* in five (31%) falcons, *M. buteonis* in five birds (three falcons, one buzzard, one harrier), and *M. gypis* in three (19%) buzzards. Identities of the amplificons were confirmed by restriction enzyme analysis. Although PCR-positive using the genus specific PCR, the *Mycoplasma* spp. detected in all Northern Goshawks and the Barn Owl could not be further identified using species-specific

TABLE 2. Distribution of *Mycoplasma* spp. from raptor nestlings, detected by culture and immunobinding assay (IBA), and by polymerase chain reaction (PCR).

Nest no.	Species[a]	Differentiation by IBA	PCR-positive result[b]
1	Northern Goshawk	Not identified[c]	*M. gypis*
	Northern Goshawk	Not identified[c]	Negative[d]
	Northern Goshawk	Contaminated sample	Negative[b]
2	Northern Goshawk	Contaminated sample	*M. gypis*
3	Northern Goshawk	Not identified[c]	Negative[b]
	Northern Goshawk	Not identified[c]	Negative[b]
	Northern Goshawk	Not identified[c]	Negative[b]
4	Eurasian Buzzard	Not identified[c]	Negative[b]
	Eurasian Buzzard	Not identified[c]	Negative[b]
5	Eurasian Buzzard	*M. gypis*	*M. gypis*
		Not identified[c]	
	Eurasian Buzzard	*M. gypis*	*M. gypis*
		Not identified[c]	
6	Red Kite	*M. gypis*	*M. gypis*
	Red Kite	*M. gypis*	*M. gypis*
7	Red Kite	*M. gypis*	*M. gypis*
	Red Kite	*M. gypis*	*M. gypis*
		Not identified[c]	
	Red Kite	*M. gypis*	*M. gypis*
8	Western Marsh Harrier	*M. gypis*	*M. gypis*
		Not identified[c]	
	Western Marsh Harrier	*M. gypis*	*M. gypis*
		Not identified[c]	
	Western Marsh Harrier	*M. gypis, M. buteonis*	*M. gypis,*
		Not identified[c]	*M. buteonis*
	Western Marsh Harrier	*M. gypis*	M. gypis
		Not identified[c]	
9	Western Marsh Harrier	Not identified[c]	Negative[b]
	Western Marsh Harrier	Not identified[c]	Negative[b]
	Western Marsh Harrier	Not identified[c]	Negative[b]

[a] Northern Goshawk (*Accipiter gentilis*); Eurasian Buzzard (*Buteo buteo*); Red Kite (*Milvus Milvus*); Western Marsh Harrier (*Circus aeroginosus*).

[b] The *Mycoplasma* genus-specific PCR (Lierz et al., 2007) was positive in all of the birds; species-specific PCR tests for the detection of 16S rRNA gene of *M. buteonis*, *M. corogypsi*, *M. falconis*, and *M. gypis* were used (Lierz et al., 2008). Only the positive results are provided; the other PCR reactions were negative.

[c] Not identified with the IBA using antisera against *M. anseris*, *M. buteonis*, *M. corogypsi*, *M. falconis*, *M. gallisepticum*, *M. gypis*, *M. iowae*, *M. lipofaciens*, *M. meleagridis*, and *M. synoviae.*

[d] Negative in all *Mycoplamsa* spp.-specific PCR tests used.

PCR tests. None of the birds tested PCR-positive for *M. corogypsi*.

DISCUSSION

Mycoplasmas are important pathogens of domestic poultry and have been suggested as a cause of disease in aviary birds and raptors (Furr et al., 1977; Gylstorff and Grimm, 1987; Gerlach, 1994; Heidenreich, 1997; Morishita et al., 1997). However, they have also been isolated from healthy raptors, indicating that some of these *Mycopasma* spp. are commensal rather than pathogenic (Poveda et al., 1990; Lierz et al., 2000, 2002, 2008).

In the present study, mycoplasmas were cultured from 91% of nestlings and from 94% of adult birds. Similarly high incidences of mycoplasma infection have been reported from raptor nestlings found injured or debilitated in the same area in Germany (Lierz et al., 2000), as well as from captive falcons in the Middle East

(Lierz et al., 2002) and captive birds of prey from Germany (Lierz et al., 2008). These high infection rates are supported by the results of the *Mycoplasma*-specific PCR; *Mycoplasma* spp. DNA was detected in tracheal swabs from all adult birds examined in this study and in healthy nestlings sampled randomly during a routine ringing program. These results support the idea that some *Mycoplasma* spp. may be commensal bacteria of the tracheal mucosa of raptors; based on these observations, the potential impact of mycoplasmosis in raptors must be re-evaluated. In the past, the demonstration of mycoplasmas from a raptor with respiratory disease usually led to the diagnosis of mycoplasmosis (Furr et al., 1977; Poveda et al., 1990; Morishita et al., 1997). Understanding the high incidence of *Mycoplasma* spp. in raptors underlines the importance of not only demonstrating the organism, but also of differentiating the species, evaluating the pathology, and conducting additional tests to exclude or identify other potential pathogens.

Mycoplasma culture is often impacted by the growth of contaminating bacteria and fungi (Lierz et al., 2000, 2002). This problem can be largely overcome by the use of a *Mycoplasma*-specific PCR (Van Kuppleveld et al., 1992; Lierz et al., 2007). The use of species-specific PCR tests also provides more detailed information about *Mycoplasma* spp. within a raptor population, especially in the case of mixed infections.

In the present investigation, using IBA, 11 isolates from raptor nestlings were identified as *M. gypis*. The results were confirmed using the *M. gypis*-specific PCR on the tracheal swabs of the same birds. This PCR also detected two additional birds as positive for this species. These findings underline the importance of using PCR when evaluating the prevalence of any *Mycoplasma* sp. within a raptor population. Some mycoplasma may overgrow others in culture, leading to false negative results. Our PCR results indicate that 57% of the examined raptors were positive for *M. gypis,* and therefore, suggest that this mycoplasma may constitute part of the commensal tracheal flora of different raptors species (Morishita et al., 1997; Lierz et al., 2000, 2008).

Compared to *M. gypis*, *M. buteonis* seemed to be less common, as it was only demonstrated in a single nestling and four adult birds; however, it was demonstrated from healthy birds, as previously reported (Lierz et al., 2002, 2008). Therefore, the pathogenic potential of this mycoplasma seems to be low, in contradiction to previous discussions (Lierz et al., 2000).

As described in earlier studies (Lierz et al., 2000, 2002, 2008) and confirmed here, *M. falconis* appears to be restricted to Falconidae. Consistent with previous studies of raptors in Germany (Lierz et al., 2000, 2008), *M. corogypsi* was not identified by culture or by PCR techniques. To date, *M. corogypsi* has only been isolated from foot-pad lesions of different raptors as well as falcons in the United Arab Emirates (Panangala et al., 1993; Morishita et al., 1997; Lierz et al., 2002), and its absence from the raptor population investigated in Germany indicates possible geographic restriction of this particular *Mycoplasma* sp. Further investigations are necessary to clarify the geographic distribution of this, and other, *Mycoplasma* spp. in raptors.

It is not uncommon to isolate or detect unidentifiable mycoplasma from raptors (Poveda et al., 1990; Lierz et al., 2000, 2002, 2008); this situation may indicate the presence of one or more undescribed *Mycoplasma* spp. or it may reflect problems with antisera specificity. Antisera directed against a reference strain may not react with another strain of the same *Mycoplasma* sp., or the species may not be represented in the antisera being used for identification. In this study, most of the *Mycoplasma* spp. detected from Northern Goshawks could not be identified to species, and further studies to identify these strains are necessary.

Unidentified mycoplasmas were also found in eight positive cultures wherein *M. gypis* was isolated. Some colonies from these plates reacted with *M. gypis* antiserum, while others did not. Using only the species-specific PCR tests, the nonidentified mycoplasma would have been missed because the *Mycoplasma*-specific PCR, as well as the *M. gypis*-specific PCR, gave a positive result. Therefore, in avian species where there is only limited knowledge of the possible *Mycoplasma* spp. that may be present, culture is still a very important tool.

To our knowledge, this study is the first to report the prevalence of mycoplasma in healthy, free-ranging raptor nestlings and the first to report the occurrence of *Mycoplasma* spp. in a Eurasian Hobby (*Falco subbuteo*), Western Marsh Harriers, and in a Barn Owl (*Tyto alba*).

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