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## SEROEPIZOOTIOLOGY OF SELECTED INFECTIOUS DISEASE AGENTS IN FREE-LIVING BIRDS OF PREY IN GERMANY

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**ABSTRACT:** Four hundred forty-eight blood plasma samples from free-living birds of prey from Berlin and the Brandenburg area in eastern Germany were tested for antibodies against Newcastle disease virus (NDV), falcon herpesvirus (FHV), owl herpesvirus (OHV), and *Chlamydia psittaci*. Antibodies to NDV were detected in 6 (2%) of 346 tested diurnal birds of prey, whereas none of the owls ( $n = 55$ ) was positive. The positive samples originated from two common buzzards (*Buteo buteo*), three ospreys (*Pandion haliaetus*) and one marsh harrier (*Circus aeruginosus*). Titers varied between 1:8 and 1:32. Of 253 birds of prey one osprey (<1%) tested positive for antibodies to FHV with low titer of 1:6. This is the first detection of antibodies against FHV in an osprey. Furthermore, antibodies against OHV could be found in one tawny owl (*Strix aluco*) and one common buzzard (2 of 253, 1%) with low titers of 1:6. Of 422 birds of prey 267 (63%) tested positive for antibodies to *Chlamydia psittaci* with titers varying between 1:5 and 1:256 which reflects the ubiquitous occurrence of *Chlamydia psittaci* in these birds of prey.

**Key words:** *Chlamydia psittaci*, falcon herpesvirus, free-living birds of prey, Newcastle disease virus, owl herpesvirus, serosurvey.

### INTRODUCTION

Newcastle disease (ND) and herpesvirus infections are important viral diseases of raptors (Forbes and Simpson, 1997). Infections with *Chlamydia psittaci* in birds of prey have been documented several times (e.g., Keymer, 1972; Gerbermann and Korbel, 1993). However, there is only limited information on the occurrence of these diseases in free-living birds of prey in eastern Germany.

Newcastle disease is caused by the avian paramyxovirus type 1 (PMV-1) in the family *Paramyxoviridae*. The pathogenicity of PMV-1 varies with both the strain of virus and host species (Manvell et al., 1997). Several species of raptors are known to be susceptible to Newcastle disease virus (NDV) (Kaleta and Baldauf, 1988) and the disease has been documented in free-living birds of prey (e.g., Keymer and Dawson, 1971; Heidenreich, 1977). According to Heidenreich (1978) hawks and harriers (Accipitridae) show a subacute to chronic course including disorders of the central nervous system, diarrhea and inappetence. An acute and fatal course has been docu-

mented in falcons (Falconidae) and owls (Strigiformes). An inapparent infection can be observed in vultures (Aegypiinae and Cathartidae) (Heidenreich, 1978). Incoordination, and therefore reduced responsiveness and ability to fly, may result from nervous disorders and free-living birds of prey are probably no longer able to catch their prey and starve (Heidenreich, 1978). Furthermore, these birds are more likely to become injured (Grimm, 1978). Newcastle disease is still active in Germany, with 209 outbreaks between 1994 and 1996 (OIE, pers. comm.). Newcastle disease virus has been found in several captive and free-living birds of prey in Germany (e.g., Winteroll, 1976; Grimm, 1978) and antibodies against NDV have been detected in 22 of 262 sera of wild fowl (26 species) in the Brandenburg area (Ziedler and Hlinak, 1993).

Herpesviral hepatitis has been reported in owls (Burtscher and Sibalin, 1975; Gough et al., 1995) and falcons (Mare and Graham, 1973; Gough et al., 1993). Owl herpesvirus (OHV) and falcon herpesvirus (FHV) are antigenetically similar and clas-

sified as  $\beta$ -herpesviruses within the family of avian Herpesviridae (Kaleta, 1990). Owl herpesvirus has been reported occurring naturally in several species of owls (Burtscher and Sibalin, 1975). Antibodies against OHV have been detected in several strigiform species (Kaleta and Drüner, 1976) as well as in falconiform birds (Kaleta and Drüner, 1976; Heidenreich and Kaleta, 1978). The disease caused by this agent can be summarized as hepatosplenitis infectiosa strigum (HSiS) (Kaleta, 1990) and in most cases a peracute to acute course can be observed (Kaleta and Drüner, 1976; Schröder, 1992). Clinical signs in owls include anorexia, depression and weakness lasting two to five days. In captivity, mortality reaches 100% and any birds surviving infection should be considered to be latently infected and likely to shed virus from time to time (Forbes and Simpson, 1997). In Germany, HSiS in raptors has previously been described (Barkhoff, 1987; Schröder, 1992). Falcon herpesvirus infections in raptors also have been documented in Europe (e.g., Gough et al., 1993; Mozos et al., 1994) and western Germany (Sander, 1995) and not only members of the family Falconidae have been found to be susceptible to FHV (Mare, 1975). Clinically FHV produces cases of inclusion body disease of falcons (Graham et al., 1975) and the course of the disease is short (24 to 72 hr) and fatal (Mozos et al., 1994). Clinical signs include anorexia, weakness, and listlessness.

Chlamydiosis is caused by *C. psittaci* and occurs worldwide. Numerous strains have been isolated from a wide range of avian and mammalian hosts (Meyer, 1967; Burkhart and Page, 1971) and at least five avian serovars have been detected (Anderson, 1991; Vanrompay et al., 1993). There are considerable differences in susceptibility of various host species to Chlamydia (Gerlach, 1994). Strains isolated from wild birds are not normally pathogenic to them as hosts but can be highly pathogenic to other avian species and humans (Brand, 1989). However, a cross-species infection

does not necessarily occur (Fowler et al., 1990). In wild as well as in captive birds the infection is most often inapparent, but the agents are still shed in the feces. Under certain circumstances such as stress or bad condition of the host, the agent can cause clinical signs or illness (Fowler et al., 1990). In raptors an apparent disease takes a chronic course and clinical signs observed are diarrhea, anorexia, lacrimation and nasal discharge (Heidenreich, 1995). Adult birds often show an inapparent infection while in young birds an acute often fatal course of the disease can be observed (Gerlach, 1994). Chlamydiosis is still present in birds in Germany, with 1,111 outbreaks between 1994 and 1997 (OIE, pers. comm.). Furthermore, the disease has been reported in captive raptors in Germany (Sabisch, 1977; Gerbermann et al., 1990) and Gerbermann and Korbel (1993) detected *C. psittaci* antigen in 13% and *C. psittaci* antibodies in 85% of free-living birds of prey in southern Germany.

Our objective in the present study was to determine the antibody prevalence against NDV, OHV, FHV and *C. psittaci* in free-living birds of prey in eastern parts of Germany (Berlin and Brandenburg). Moreover, we were interested to know to what extent rare species of birds of prey such as the peregrine falcon (*Falco peregrinus*), osprey (*Pandion haliaetus*) and the white-tailed sea eagle (*Haliaeetus albicilla*) have been exposed to these agents.

#### MATERIAL AND METHODS

In collaboration with various raptor rehabilitation centers (Fig. 1) 428 blood plasma samples from free-living birds of prey were collected between 1994 and 1997. The samples originated from areas in Berlin (52°30'N, 13°20'E) and the Brandenburg region (51°35' to 53°45'N, 11°60' to 14°70'E) in eastern Germany. The samples were from different raptor species (Table 1).

In general, blood plasma samples were either obtained from apparently healthy birds during ringing procedures or from injured or sick birds during the clinical examination at the raptor centers. Blood was usually taken within 48 hr after the bird was taken to the raptor

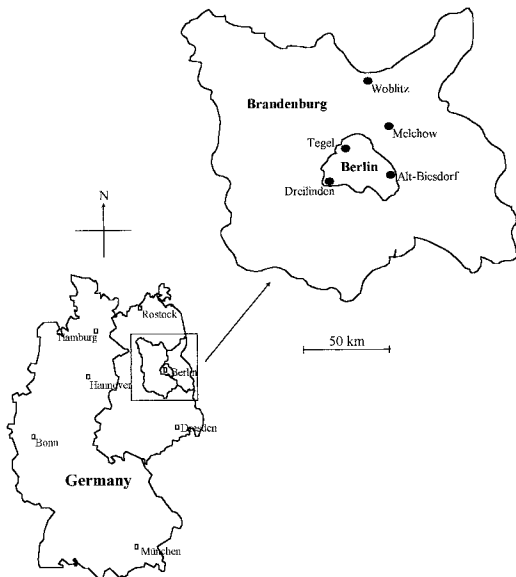


FIGURE 1. Distribution of the collaborating raptor rehabilitation centers in the Berlin and Brandenburg area in eastern Germany.

center. In most cases age (nestlings, juveniles, subadults and adults) and sex of the bird was known. Samples were stored at  $-20\text{ C}$  until examined.

Blood plasma samples were tested for antibodies against NDV (La Sota strain) by hemagglutination-inhibition (HI) test (Kaleta et al., 1972). HI test was performed in 'V' bottomed microtiter plates using twofold dilutions of plasma, 1% chicken red blood cells and 4 hemagglutinating units (HA) of antigen in 0.025 ml volumes. All samples were diluted 1:8 before use in the HI test. Titers  $\geq 1:8$  were considered positive (Mayr et al., 1977).

Furthermore, blood plasma samples were tested for antibodies against OHV and FHV by virus neutralisation test (NT) (Sander, 1995). A FHV-isolate from a hybrid falcon (kindly provided by C. Grund; Ludwig Maximilian University, Munich, Germany), and an OHV-isolate 473/73 (kindly provided by E. F. Kaleta, Justus Liebig University, Giessen, Germany) were used in the NT for the detection of anti-FHV and anti-OHV antibodies, respectively. The NT was performed in 96-well microtiter plates (Nunc, Gibco, Paisley, Renfrewshire, UK) using 100 TCID<sub>50</sub> (dose infecting 50% of the inoculated tissue culture cell) FHV and OHV per 100  $\mu\text{l}$  well and twofold plasma dilution. The antigen/plasma mixture (each 25  $\mu\text{l}$  per well) was incubated for 1 hr at 37 C. Subsequently,  $4 \times 10^5$  cells/ml (50  $\mu\text{l}$ ) were seeded into each well. Primary cultures of chicken embryo fibro-

blasts (CEF) and Dulbecco's modified eagle medium supplemented with 2% fetal bovine serum (FBS) were used for verification of FHV and OHV. The CEF were prepared from 11-day-old chicken embryos by using conventional methods (Schmidt, 1964). All media contained 10% tryptose and 0.5% gentamycin. For 7 days the CEF cell-cultures were examined for cytopathic effects. Titers were calculated according to Spearman and Kärber (1985). Titers  $\geq 1:4$  were considered positive (Schröder, 1992).

Blood plasma samples were tested for antibodies against *C. psittaci* using a commercial competitive enzyme-linked immunosorbent assay (R-Biopharm GmbH, 64293 Darmstadt, Germany). This test has been developed for detection of *C. psittaci* antibodies in birds independently of the species. All samples were tested at a dilution of 1:5 and subsequently all positive samples were titrated at 1:10, 1:16, 1:32, 1:64, 1:128 and 1:256 dilutions. The percentage inhibition (PI) of a sample was evaluated from its extinction value (E) and the mean extinction value of the negative controls (EC) ( $\text{PI} = 100 - \text{E}/\text{EC}$ ). An PI of  $\geq 20\%$  was considered positive.

A stepwise logistic regression procedure (Hosmer and Lemeshow, 1989) was performed to evaluate the potential influence of multiple variables on the seroprevalence for *C. psittaci*. In this study the species ( $n = 422$ ), age ( $n = 364$ ) and sex ( $n = 223$ ) of a bird and their first order interaction effects were used as independent variables. Interdependencies between pairs of categorical or binary variables were tested by the chi-square test (Bortz et al., 1990) or, in case of low frequencies, by its exact version. Adjusted standardized residuals in contingency tables were calculated to identify the categories responsible for significant chi-square values (Everitt, 1977). Pfanzagl's test (Bortz et al., 1990) was used to verify a potential monotonous trend in a  $r \times 2$  contingency table. The significance level was set to  $\alpha = 0.05$ . All statistical calculations (except Pfanzagl's test) were performed using the SPSS version 9.0 software.

## RESULTS

Of 346 diurnal birds of prey, six (2%, 95% confidence interval [CI] 0.6–3.7) showed antibodies against NDV whereas none of the tested owls ( $n = 55$ ) was positive. The positive samples originated from two common buzzards, three ospreys and one marsh harrier (Table 1) with titers varying between 1:8 and 1:32. The buzzards and the marsh harrier showed clini-

TABLE 1. Antibodies to Newcastle disease virus, falcon herpesvirus, owl herpesvirus, and Chlamydia psittaci.

Species	NDV <sup>a</sup>	FHV <sup>b</sup>	OHV <sup>c</sup>	C. psittaci <sup>d</sup>
Common buzzard ( <i>Buteo buteo</i> )	2/110 [CI 0.00–0.06] <sup>e</sup>	0/88	1/88 [CI 0.00–0.06]	75/111 [CI 0.58–0.76]
Eurasian kestrel ( <i>Falco tinnunculus</i> )	0/75	0/31	0/31	49/82 [CI 0.48–0.70]
Goshawk ( <i>Accipiter gentilis</i> )	0/46	0/32	0/32	28/48 [CI 0.43–0.72]
European hobby ( <i>Falco subbuteo</i> )	0/26	0/16	0/16	21/29 [CI 0.53–0.87]
Tawny owl ( <i>Strix aluco</i> )	0/24	0/17	1/17 [CI 0.00–0.29]	21/26 [CI 0.61–0.93]
Osprey ( <i>Pandion haliaetus</i> )	3/20 [CI 0.03–0.38]	1/8 [CI 0.00–0.53]	0/8	13/20 [CI 0.41–0.85]
Eurasian sparrowhawk ( <i>Accipiter nisus</i> )	0/18	0/3	0/3	13/24 [CI 0.33–0.74]
Long eared owl ( <i>Asio otus</i> )	0/14	0/8	0/8	12/14 [CI 0.57–0.98]
Red kite ( <i>Milvus milvus</i> )	0/13	0/9	0/9	3/14 [CI 0.05–0.51]
Barn owl ( <i>Tyto alba</i> )	0/12	0/5	0/5	8/11 [CI 0.39–0.94]
Black kite ( <i>Milvus migrans</i> )	0/8	0/4	0/4	3/8 [CI 0.09–0.76]
White-tailed sea eagle ( <i>Haliaeetus albicilla</i> )	0/7	0/6	0/6	7/7 [CI 0.59–1.00]
Northern rough legged buzzard ( <i>Buteo lagopus</i> )	0/7	0/5	0/5	3/7 [CI 0.10–0.82]
Peregrine falcon ( <i>Falco peregrinus</i> )	0/6	0/5	0/5	1/6 [CI 0.00–0.64]
Honey buzzard ( <i>Perisoreus inornatus</i> )	0/5	0/4	0/4	4/4 [CI 0.40–1.00]
Eagle owl ( <i>Bubo bubo</i> )	0/4	0/3	0/3	1/4 [CI 0.01–0.81]
Marsh harrier ( <i>Circus aeruginosus</i> )	1/4 [CI 0.01–0.81]	0/3	0/3	4/4 [CI 0.40–1.00]
Merlin ( <i>Falco columbarius</i> )	0/1	—	—	0/1
Tengmalm's owl ( <i>Aegolius funereus</i> )	0/1	0/5	0/5	0/1
Hen harrier ( <i>Circus cyaneus</i> )	— <sup>f</sup>	0/1	0/1	1/1 [CI 0.03–1.00]
Total	6/401	1/253	2/253	267/422

<sup>a</sup> Newcastle disease virus.

<sup>b</sup> Falcon herpesvirus.

<sup>c</sup> Owl herpesvirus.

<sup>d</sup> Chlamydia psittaci.

<sup>e</sup> Number of positive reactors/number of samples tested [CI 95% confidence interval].

<sup>f</sup> Insufficient volume available.

cal signs such as paresis of the pelvic limbs, inappetence and cachexia. However, the three positive ospreys showed no clinical signs and all three of them were breeding at the stage of blood sampling. The seroprevalence varied in the three different years. In 1995 one of 154 (<1%, CI 0.0–3.6), 1996 four of 192 (2%, CI 0.6–5.2) and 1997 one of 49 (2%, CI 0.1–10.9) birds of prey showed antibodies against NDV.

Of 253 birds of prey, one osprey (<1%, CI 0.0–2.2) had antibodies against FHV (Table 1) with a low titer of 1:6. The sample originated from a breeding adult, male bird. The bird was apparently healthy at the time of blood taking. In two (1%, CI 0.1–2.8) of 253 birds of prey antibodies against OHV were detected with low titers of 1:6. One of the samples originated from an adult tawny owl (Table 1). The bird showed no clinical signs except a poor state of nutrition. The other positive sample originated from an adult, female common buzzard that had to be euthanized due to a spine fracture. Pathological findings were hepatitis and air sacculitis.

Antibodies against *C. psittaci* were detected in 267 of 422 samples (63%, CI 58.5–67.9, Table 1) with titers varying between 1:5 and 1:256. According to stepwise logistic regression only age proved to influence the seroprevalence significantly ( $P = 0.0019$ ,  $n = 213$ ). However, due to incomplete sex and age data the logistic regression could only make limited use of the test results (213 of 422). Therefore, species, age and sex differences of seroprevalence were also tested separately to include more information. One hundred and thirty-seven of 180 adult (76%, CI 69.2–82.1), 13 of 22 subadult (59%, CI 36.4–79.3), 48 of 84 juvenile (57%, CI 45.9–67.9) and 30 of 81 nestling birds (37%, CI 26.6–48.5) were positive. These differences are statistically significant ( $P < 0.001$ ,  $n = 364$ ). Furthermore, a monotonous trend from lower seroprevalences for younger birds up to higher seroprevalences for older birds could be statistically con-

firmed (Pfanzagl's test,  $P < 0.0001$ ,  $n = 364$ ). Regarding the sex no significant difference was found ( $P = 0.586$ ,  $n = 223$ ). Apparently, the percentage of positive reactors varied between different raptor species. However, as there are interdependencies between age and species in this study ( $P < 0.001$ ,  $n = 365$ ) these potential differences were examined separately for each age group. Significant differences in seroprevalence between different species were only found within the group of nestling birds ( $P = 0.005$ ,  $n = 81$ ), but not among adults ( $P = 0.176$ ,  $n = 176$ ), subadults ( $P = 0.693$ ,  $n = 22$ ) and juveniles ( $P = 0.125$ ,  $n = 83$ ). These differences are due to common buzzard nestlings showing slightly more positive individuals than expected (adjusted standardized residual = 3.6). No significant differences in seroprevalence between strigiform birds and falconiform birds were observed (adults:  $P = 0.112$ ,  $n = 176$ ; subadults: no data available; juveniles:  $P = 0.693$ ,  $n = 83$ ; nestlings:  $P = 0.215$ ,  $n = 81$ ).

#### DISCUSSION

This serological survey reveals the evidence for a natural exposure to NDV, FHV, OHV and *C. psittaci* in free-living raptors in the Berlin and Brandenburg region in eastern Germany. Antibodies to NDV were detected in plasma samples from six diurnal birds of prey. Different from previous descriptions (Zuydam, 1952) the positive ospreys showed no clinical signs and were breeding at the stage of blood sampling. It is most likely that in this case the disease must have taken a mild or inapparent course with persistent antibodies. The remaining three birds showed clinical signs such as paresis of the pelvic limbs, inappetence and cachexia, which have been described in raptors with ND (Winteroll, 1976; Forbes and Simpson, 1997). In our case it remains uncertain if these clinical signs are connected to ND because no virus was isolated. Infections with NDV (of all species) have recently been limited to the less velogenic

forms causing mild disease with transient nervous signs from which the birds recover completely and low mortality (Forbes and Simpson, 1997) as described in this study. None of the 55 tested owls showed antibodies against NDV, probably because ND in owls takes an acute and fatal course (Heidenreich, 1978) during which these birds are often not able to produce antibodies (Winteroll, 1976). Lower antibody prevalence rate to NDV was observed in 1995 (<1%) compared to 1996 and 1997 (2%). This indicates that there are periodical differences regarding the exposure to NDV in birds of prey in Germany. The incidence of ND seems to have increased in Europe since 1991 (Alexander, 1995). A potential threat to rare species of birds of prey by ND can not be excluded even though in our case the disease has obviously not been a serious threat to the three ospreys.

In one osprey antibodies against FHV were found. This is the first detection of antibodies against FHV in an osprey. Our results indicate that the free-living osprey had been exposed to FHV without showing the acute and fatal disease as known in falcons (Mozos et al., 1994). Nevertheless, a potential threat to rare species of raptors by FHV in this region can not be excluded. Two birds of prey had been exposed to OHV although only low titers were detected. One positive reactor was a tawny owl. Antibodies against OHV in apparently healthy owls from western Germany, including seven tawny owls, have been detected before (Kaleta and Drüner, 1976). Furthermore, antibodies were found in a common buzzard that had hepatitis. Heidenreich and Kaleta (1978) demonstrated that after an experimental infection with OHV common buzzards produced antibodies for a short period of 7 to 14 days without showing any clinical signs. In our case it remains uncertain if the clinical signs observed in the common buzzard are related to an OHV infection.

In 63% of the birds antibodies against *C. psittaci* were detected. These results

are in agreement with investigations of Gerbermann and Korbel (1993) in free-living birds of prey in southern Germany. Our investigations reflect the ubiquitous occurrence of *C. psittaci* in birds of prey in the Berlin and Brandenburg area. A positive correlation between age and the number of positive reactors could be found. This suggests that the probability of an immunological response to the pathogen increases with age. Otherwise, this might indicate that the mortality rate due to *C. psittaci* in nestlings and juvenile birds is higher than in subadult or adult birds, which would conform with knowledge about the clinical picture of chlamydiosis (Gylstorff and Grimm, 1998). In accordance with investigations from Gerbermann and Korbel (1993) the percentage of positive reactors varied between different raptor species. However, in this study these differences are not due to the species but to the age except within the group of nestlings in which buzzards show a slightly higher seroprevalence than expected. It remains unknown which factor is responsible for this difference. However, it might be explained by different hunting and feeding behaviour. Common buzzards are known to have a broad spectrum of prey, including carrion, and poor nest hygiene (Hastädt and Sömmer, 1987; Glutz von Blotzheim et al., 1989).

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