Raptors in WNV Epidemiology

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West Nile virus exists in a natural cycle between mosquitoes and wild birds, with humans and horses acting as dead-end hosts. Avian species are key in WNV transmission cycle and, therefore, have been the focus of surveillance across many countries. Raptors appear particularly susceptible to WNV infection, resulting in higher prevalence, resulting in clinical disease and death. Birds of prey are known to play an important role as WNV reservoir and potentially act as amplifying hosts of infection. Importantly, raptor higher susceptibility/prevalence may indicate infection through predation of infected prey. Consequently, becoming and important target species when designing cost-effective surveillance for monitoring both seasonal WNV circulation in endemic countries and its emergence into new areas, even outside the mosquito season.

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1. Introduction

West Nile virus (WNV) is a zoonotic agent that is maintained in a transmission cycle between birds and mosquitoes. WNV is member of the genus *Flavivirus* of the family *Flaviviridae*. WNV strains are classified into at least 7 genetic lineages[1][2] with highly pathogenic isolates mainly belonging to lineage 1 (L1) and 2 (L2) strains [3]. It causes a febrile disease in humans and horses that in some cases progresses to fatal encephalitis. Even though several human vaccine candidates have been developed for WNV, none has been licensed and currently there are no available WNV vaccines for humans[4].

WNV L1 was first isolated in Africa[5][6] from where it spread to the Middle East and Europe during the mid-twentieth century, and finally to the United States (US) in 1999[7][8][9]. Since 2004, a new WNV L2 is circulating in Europe[1][10] and is responsible for the unprecedented increase of WNV human cases registered since 2018 [11][12][13]. In Europe, outbreaks in human and equine populations have usually been documented in Mediterranean countries. However, new WNV cases have recently been reported in humans, avian, and equine species in Germany at higher latitudes than in previous years[14].

As the key vertebrate hosts in WNV transmission cycle, avian species are the focus of surveillance across the world [15][16][17]. Birds of prey are target species in these surveillance programs because some species are very susceptible to infection and exhibit a wide range of clinical signs. In fact, WNV infection is the most frequently diagnosed infectious disease among raptors in the US and Canada[17][18][19].

Birds of prey or raptors, derived from the latin verb *rapio* (to seize), are those predatory birds that catch prey using sharp talons, or claws, for grasping and killing prey, and a powerful curved beak for tearing flesh. The group is not taxonomically valid but includes members from the orders Accipitriformes (eagles, ospreys, kites, true hawks, buzzards, harriers, and vultures), Falconiformes (falcons and caracaras) and Strigiformes (owls). This large group of birds are found throughout the world. WNV is transmitted to raptors mainly through mosquito bites. However, oral transmission through the consumption of infected prey or carrion has also been described[20].

One of the main challenges when dealing with diseases of wildlife is the limited access to the animals of interest. When wildlife becomes infected, determining the clinical response and outcomes are difficult to establish when such events are seldom observed. In the case of birds of prey and WNV, some of these questions have been addressed using (i) observations in captive birds usually from wildlife rehabilitation centers (WRC), (ii) active surveillance programs, and (iii) experimental infections.

2. Infection and Immunity

2.1. Infection in Birds of Prey

Observing clinical signs in wildlife is challenging, given that wild birds are often difficult to observe and sparsely distributed. Detecting disease is often easier in domestic animals, but in the case of WNV most poultry species, such as the chicken (*Gallus gallus*), appear refractory to overt clinical disease. The exception is the domestic goose (*Anser anser domesticus*), where WNV-associated mortality has been previously reported[21][22][23].

For birds of prey, the pathogen is usually detected in the carcasses of birds found dead or moribund through wildlife surveillance programs, raptors admitted to WRC[24], or following mass mortality events[20]. One raptor species in Europe that has repeatedly been associated with WNV infection is the Northern goshawk (*Accipiter gentilis*)[17][18][25][26][27][28]. The reason for this is unclear, although the increased incidence observed in Northern goshawks (*Accipiter gentilis*) may be associated to the species predation on smaller birds that can also act as a reservoir for WNV, as demonstrated by experimental raptor infection through feeding of WNV-infected prey[29].

In most cases, evidence for WNV-associated disease is obtained in wild dead birds on pathological investigation. In addition, confirmation of WNV infection may occur following the observation of clinical disease in captive birds. For example, in Canada, shortly after the introduction of WNV in the country, a large number of captive North American owls (108 out of 245) died at a WRC in Ontario[30]. Necropsy samples of brain, lung, liver, and spleen from 85 birds were tested and 79 were confirmed WNV positive by RT-PCR. Again, captive raptors, both those born and raised in captivity, and those that are being rehabilitated, can form a cornerstone of a WNV surveillance program due to their susceptibility to infection[17].

A small number of experimental infections have been attempted on raptor species and have reported mixed findings with respect to clinical disease and infection outcomes. A large study of five different raptor species including the American kestrel (*Falco sparverus*), Golden eagle (*Aquila chrysaetos*), Red-tailed hawk (*Buteo* *jamaicensis*), Barn owls (*Typto alba*) and Great-horned owls (*Bubo virginianus*) assessed three methods of WNV infection routes: oral infection with infected mice; infected mosquitoes; and direct needle inoculation with an L1 strain. However, no clinical signs were observed in this study or in experimentally infected Gyrfalcons (*Falco rusticolus*)[31][32]. In contrast, experimental infection of large falcons resulted in neurological disease and death after subcutaneous needle inoculation with higher challenge doses of an L1 strain and after inoculation with different doses of an L2 strain[33]. Another factor that may influence the outcome of WNV infection in raptors is the particular species-specific susceptibility. In general, owls appear to be more likely to develop neurological clinical signs than other raptors, and among owls, those from northern species, such as the Great Horned owl (*Bubo virginianus*) and Snowy owl (*Bubo scandiacus*), seem more susceptible[30]. However, statistically significant differences in mortality rates and WNV prevalence have not been found among taxonomic orders, age class, or sex, with the exception of immature Red-tailed hawks (*Buteo jamaicensis*), which were found to be more susceptible than adult Red-tailed hawks[34].

2.2. Immunity against WNV in Birds of Prey

In birds, as in mammals, protection against WNV is determined by the presence of antibodies in the blood of the individual. This can be measured by a range of serological assays, the most stringent being a virus neutralization test (VNT), which detects serum neutralizing antibodies and more accurately detects protective antibodies. The main alternative is the capture enzyme-linked immunoassay (ELISA) that detects antibodies directed against the virus envelope protein. Also, there are class-specific ELISAs for the detection of WNV Immunoglobulin (Ig) Y (the avian equivalent of IgG in mammals) or IgM. In adult birds, antibodies are developed following exposure to, and infection with WNV. The detection of such antibodies in an apparently healthy bird suggests either past asymptomatic infection or recovery from a non-fatal infection[35]. Seropositive birds are likely protected against infection in future exposure to WNV, as assayed in experimental studies in some bird species such as the House sparrow (*Passer domesticus*)[36]. However, no experimental data on long-term humoral immunity duration is available for raptors.

Longitudinal sampling of raptors held in WRC has confirmed the seroconversion of birds of prey in response to the seasonal emergence of WNV in North America[37]. Different studies have detected seropositive birds of prey in both Europe[38][39] and the Americas[40][41]. These findings suggest past WNV infection but recovery in otherwise healthy birds. Experimental infections in raptors have consistently shown the induction of anti-WNV antibodies, both total and neutralizing antibodies, from 6 dpi[31][32][33]. The exception to this is the presence of maternal antibodies in juvenile birds. IgY is present in the egg yolk and crosses the yolk sac membrane to enter the bloodstream of the developing embryo. If the mother has developed anti-WNV antibodies, these will be transferred conferring temporary immunity to the young. In raptors, maternal transfer of WNV neutralizing antibodies was demonstrated in a captive breeding colony of Eastern screech-owls (*Megaschops asio*)[42]. However, subsequent studies in the House sparrow suggest that maternally-derived antibodies decline rapidly, being undetectable after 9 days, and do not confer protection to the juvenile after this point[43]. Maternal antibodies were suggested as a potential reason for the absence of disease in Red-footed falcon (*Falco vespertinus*) nestlings in the presence of infected mosquito vectors[44]. Avian immunity research has focused on humoral immunity, i.e., the induction of antibodies. However, there is a dearth of information on the cell-mediated responses in avian species and how these assists in controlling WNV infection.

2.3 Diagnosis and surveillance

Early WNV detection in raptor clinical cases may trigger surveillance in other animal species, maximizing the possibility of WNV detection, which may be useful when the virus is circulating at low levels. Additionally, WNV surveillance in dead birds of prey could be adequate even after the end of the period of mosquito activity, where the virus could be detected in winter, most probably transmitted via predation .

Clinical signs reported in WNV-infected raptors vary widely between species and among individuals. Interestingly, many WNV-positive raptors often show ocular lesions and evidence of trauma , as well as concurrent lesions of other non-viral infectious diseases, such as bacterial septicemia, aspergillosis and *Leucozytozoon* spp. infection . Therefore, it is important to consider concurrent WNV infection especially in birds with traumatic injuries, where WNV might have contributed to death. Gross lesions are infrequent in all raptor species in comparison to clinical signs or histopathological lesions . The most frequently described macroscopic lesion among WNV-infected raptros is emaciation or cachexia. Other frequently reported gross lesions include calvarial and meningeal hemorrhages. However, those may be related to concurrent trauma.

A variety of tissues including brain, heart, or liver can be used with success for viral detection in infected birds . In those tissues, viral genome and antigens can be detected by RT-PCR and IHC, respectively. Histopathologic lesions in raptors are more commonly observed in the heart and brain, while the heart and kidney were the organs more commonly reported to show WNV positivity by immunohistochemistry (IHC) . Besides infected tissues, choanal, oral and cloacal swabs may be used to detect the virus . It is worth mentioning that oral swabs were more sensitive to detecting viral shedding than cloacal swabs in some experimentally infected raptor species . The viral detection via molecular techniques, such as RT-PCR, allows subsequent viral sequencing and phylogenetic analysis, which may enable the identification of viral strains circulating in specific areas.

Additionally, serum samples from resident raptors can also be used to provide information of virus circulation since seroconversion or a significant increase in antibody titers in two serially collected specimen indicates recent WNV infection, as reported in Germany in 2018 .For example, clinic-admitted raptors diagnosed by both molecular and serological testing allowed the detection of virus circulation before other surveillance systems . However, serological samples and tests may be more difficult to obtain and perform that molecular techniques.

Surveillance based on molecular WNV diagnosis using swabs of raptors with general signs of illness, such as dehydration, emaciation, and debilitation with or without neurological signs, may be more feasible since samples can be easily provided by WRCs. This kind of surveillance may provide a reliable ante-mortem diagnosis of current WNV infection . Additionally, bird samples (swabs and tissues) for WNV detection could be collected and shipped using FTA™ and RNASound™ cards , which preserve the viral genome and are easier to transport. WRCs, which receive high numbers of raptors, can certainly obtain samples for WNV diagnosis from birds in a cost-effective manner in comparison with other types of surveillance such as virus testing in the mosquito population during periods of low viral transmission.

In this sense, it is worth highlighting Northern goshawks as an indicator of WNV activity and ongoing emergence, since this species has been repeatedly found dead due to WNV infection in Europe . Therefore, birds of prey from WRC should be included in all WNV surveillance programs since they can reflect the WNV-infection status of the area.

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