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Domestic goose as a model for West Nile virus vaccine efficacy

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ABSTRACT

West Nile virus (WNV) is an emergent pathogen in the Americas, first reported in New York during 1999, and has since spread across the USA, Central and South America causing neurological disease in humans, horses and some bird species, including domestic geese. No WNV vaccines are licensed in the USA for use in geese. This study reports the development of a domestic goose vaccine efficacy model, based on utilizing multiple parameters to determine protection. To test the model, 47 geese were divided in two experiments, testing five different vaccine groups and two sham groups (challenged and unchallenged). Based on the broad range of results for individual metrics between the Challenged-Sham and Unchallenged-Sham groups, the best parameters to measure protection were Clinical Pathogenicity Index (CPI), plasma virus positive geese on days 1–4 post-inoculation and plasma virus titers, and brain histological lesion rates and severity scores. Compared to the Challenged-Sham group, the fowlpox virus vectored vaccine with inserts of WNV prM and E proteins (vFP2000) provided the best protection with significant differences in all five metrics, followed by the canarypox virus vectored vaccine with inserts of WNV prM and E proteins (vCP2018) with four metrics of protection, recombinant vCP2017 with three metrics and WNV E protein with one. These data indicate that domestic geese can be used in an efficacy model for vaccine protection studies using clinical, plasma virological and brain histopathological parameters to evaluate protection against WNV challenge.

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1. Introduction

West Nile virus (WNV) is a mosquito-transmitted, single-stranded positive-sense RNA virus of the *Flaviviridae* family, genus *Flavivirus*. Disease caused by WNV was first reported in 1937 in a febrile woman in Uganda, and since then several outbreaks have been reported in humans, horses, and some birds worldwide [1]. The first reported WNV occurrence in the Americas was in 1999 in USA as an outbreak of viral encephalitis in humans with concurrent high mortality in American crows (*Corvus brachyrhynchos*) and fish crows (*Corvus ossifragus*) as well as die-offs in several exotic avian species at the Bronx Zoo [2]. The virus isolated from that outbreak was closely related to a West Nile virus isolated previously in 1998 from a dead goose in Israel [2]. After the outbreak in New York the virus spread in United States, Canada, Mexico, Central America, Caribbean, and South America [1].

Several North American bird species are reservoirs for the virus and birds in the orders Passeriformes and Charadriiformes are apparently the most efficient ones based on viremia data from experimental infections [3]. Even though many birds develop viremia and are able to transmit the virus to naïve mosquitoes, just a few bird species, like crows (*Corvus brachyrhynchos* and *Corvus ossifragus*), Chilean flamingo (*Phoenicopterus chilensis*) [2], domestic turkeys [4,5], and domestic geese [6–8] are reported to develop clinical disease in natural infection or experimental conditions. Among poultry, geese are the species mostly associated with natural WNV infection and disease [6–8]. The disease has been associated with high mortality in domestic geese in Israel [9], Canada and the USA [5,7].

There are a few commercially available vaccines to control the disease in horses, but so far no vaccine is commercially available to use in birds, even though some of the commercially available for horses are reported to be used in exotic birds species [10]. A recombinant canarypox vector (ALVAC® – vCP2017) with WNV membrane protein (prM) and envelope (E) protein was licensed in 2004 and an attenuated WNV live flavivirus chimera vaccine was licensed in 2006 for use in horses, but the last one was withdrawn

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from the market for safety reasons. Several experimental vaccine protection studies have been conducted using either killed WNV in flamingos [10,11] and other bird species such as penguins and prairie chickens [10], and in geese vaccines using Israel turkey meningoencephalitis (TME) [12] and also subunit vaccines are also reported [13]. Protection based on neutralizing serological response or challenge varied depending on the bird species and dose of vaccine [10,14].

The aim of this study was to evaluate the use of geese as an experimental model for testing WNV vaccines for efficacy using different measures of protection such as prevention of illness and death, decrease in plasma virus titer, and protection against histopathological lesions in heart and brain.

2. Materials and methods

2.1. Cells and viruses

West Nile strain 9/99 isolated from a crow in New York (courtesy of E. Ostlund, National Veterinary Services Laboratories, Ames, Iowa) was propagated in Vero cells and used as a challenge virus. Vero cells were maintained at 37 °C in an atmosphere of 5% CO_2 in MEM (Invitrogen, Carlsbad, CA) supplemented with 0.05 mg/ml of gentamicin and 10% fetal bovine serum (Invitrogen).

2.2. Geese and housing

Forty-seven 1-week-old domestic geese (*Anser anser domesticus*) (Privett Hatchery, Portales, New Mexico) were housed in positive pressure HEPA-filtered isolators located in an Animal Biosafety Level 3 Enhanced facility with feed and water provided ad libitum. The study was approved by USDA/ARS/SEPRL internal Biosafety committee. General care was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*.

2.3. Vaccines tested and experimental design

The 47 geese were divided in two experiments. Experiment one with groups 1–6, and experiment two with groups 7 and 8. Group 1, ($n = 8$) sham vaccinated and challenged (Challenged-Sham group); group 2, ($n = 5$) vaccinated with vCP2017 (ALVAC® Merial, Athens, GA, USA), a vectored canarypox virus expressing prM and E WNV proteins; group 3, ($n = 5$) vCP2018 (Merial, Athens, GA, USA), a vectored canarypox virus expressing prM and E WNV proteins; group 4, ($n = 5$) vFP2000 (Merial, Athens, GA, USA), a fowlpox virus vector expressing prM and E WNV proteins; group 5, ($n = 5$) whole virus inactivated West Nile antigen prepared with mineral oil as adjuvant (Oil-emulsion WNV group); group 6 ($n = 7$), sham vaccinated with diluent plus adjuvant and remained unchallenged (Unchallenged-Sham group). Experiment two consisted of group 7 ($n = 6$), sham vaccinated and challenged (Challenged-Sham group); and group 8 ($n = 6$) vaccinated with purified E WNV protein (E Protein group), prepared with mineral oil as adjuvant. The E protein used in group 8 vaccine was expressed in *Pichia pastoris*, and after purification was used as antigen in a oil emulsion vaccine as described bellow. The vaccines used for sham groups, whole virus inactivated antigen and E protein were prepare specifically for these studies.

The geese were vaccinated at 1 week of age and received a vaccine booster 14 days later at 3 weeks of age. The recombinant vaccines for groups 2–4 were titrated and diluted to 10^6 PFU/dose, and birds in group 8 received 50 μg of purified E protein. The vaccines for groups 2–4 were produced using an adjuvant provided by the manufacturer. Vaccines for groups 5 and 8 were mechanically emulsified in oil phase as described [15,16]. All the vaccines were administered intramuscularly into the thigh in a 0.2 ml volume.

Groups 1–5 were challenged at 14 days after the booster vaccination and group 8 was challenged seven days after booster using $10^{3.1}$ $TCID_{50}$ of WNV strain New York 1999 in a 0.2 ml volume by subcutaneous route. The challenging dose was based on previous studies with geese [8]. All surviving geese were humanely euthanized 10 days post challenge (DPI). Blood was collected for serology at day of the first vaccination, day of the booster, day of challenge and at the end of experiment 10 DPI. Blood with EDTA was also collected on 0, 1, 2, 3, 4, 5 and 7 and 10 DPI for virus isolation and titration from plasma. Brain and heart were collected for histopathological examination and immunohistochemistry from all geese on the day of death or at 10 DPI. Clinical Pathogenicity Index (CPI) was calculated as average daily score for each bird with 0 = normal, 1 = mild signs (incoordination, difficulty walking, unsteadiness or reluctance to stand), 2 = moderate clinical disease (tremors, paresis/paralysis, seizures, or inability to control head position), 3 = moribund or dead.

2.4. Virus isolation and titration

Virus isolation was performed in confluent Vero cells inoculated with 0.1 ml of plasma and observed for cytopathic effect (CPE) for 5–7 days. One blind passage was made for each of the negative samples. All positive samples were diluted and titrated in Vero cells as described previously [4].

2.5. Serology

Sera obtained pre first vaccine dose, pre booster, on the day of challenge and 10 DPI were tested by a WN specific ELISA following the protocol described by Ebel et al. [17]. Samples with OD higher than the average of the negative control plus 3 times the standard deviation of the negative controls were considered positive.

2.6. Histology and immunohistochemistry

Brain and heart were collected on the day of death or day of humane euthanasia, fixed by submersion in 10% neutral buffered formalin, and routinely processed for histological staining with hematoxylin and eosin (HE). Duplicate sections were immunohistochemically stained using a mouse polyclonal antibody that cross-reacts with several members of Japanese Encephalitis serocomplex group flavivirus using the same methodology previously described [8].

2.7. Statistical tests

The morbidity and mortality rates, virus isolation positive rates, antibody positive rates, and histopathology and immunohistochemistry rates were tested by Fisher's exact test. The histological lesion scores, immunohistochemistry scores, CPI and virus titer levels in the plasma were analyzed by Mann–Whitney test, comparing each group with the respective Challenged-Sham group for each one of the experiments. The differences were considered significant when $p < 0.05$. The analyses were performed using GraphPad Prism version 5.04 for Windows, GraphPad Software, La Jolla, CA, USA, www.graphpad.com.

3. Results

The ten measures of protection (mortality and morbidity rates, CPI, virus isolation rates and titers in plasma, antibody positive rates, histological lesion rates and scores, and immunohistochemical demonstration of viral antigen rates and scores) of groups 2–6