Response to and efficacy of vaccination against eastern equine encephalomyelitis virus in emus

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Objective—To evaluate humoral immune responses of emus vaccinated with commercially available equine polyvalent or experimental monovalent eastern equine encephalomyelitis (EEE) virus and western equine encephalomyelitis (WEE) virus vaccines and to determine whether vaccinated emus were protected against challenge with EEE virus.

Design—Cohort study.

Animals—25 emus.

Procedure—Birds were randomly assigned to groups (n = 5/group) and vaccinated with 1 of 2 commercially available polyvalent equine vaccines, a monovalent EEE virus vaccine, or a monovalent WEE virus vaccine or were not vaccinated. Neutralizing antibody responses against EEE and WEE viruses were examined at regular intervals for up to 9 months. All emus vaccinated with the equine vaccines and 2 unvaccinated control birds were challenged with EEE virus. An additional unvaccinated bird was housed with the control birds to assess the possibility of contact transmission.

Results—All 4 vaccines induced detectable neutralizing antibody titers, and all birds vaccinated with the equine vaccines were fully protected against an otherwise lethal dose of EEE virus. Unvaccinated challenged birds developed viremia (> 10⁶ plaque-forming units/ml of blood) and shed virus in feces, oral secretions, and regurgitated material. The unvaccinated pen-mate became infected in the absence of mosquito vectors, presumably as a result of direct virus transmission between birds.

Conclusions and Clinical Relevance—Results indicate that emus infected with EEE virus develop a high-titer viremia and suggest that they may serve as important virus reservoirs. Infected emus shed EEE virus in secretions and excretions, thereby posing a health risk to humans and animals. Commercially available polyvalent equine vaccines protect emus against EEE virus infection. (J Am Vet Med Assoc 2001;218:1469–1473)

Eastern equine encephalomyelitis (EEE) and western equine encephalomyelitis (WEE) viruses are clinically important zoonotic vector-borne alphaviruses. These viruses are amplified in nature through complex cycles involving birds and mosquitoes. Although EEE is considered the more virulent virus in humans and horses, both viruses can cause considerable morbidity (eg, encephalitis, meningitis, and meningoencephalitis) and mortality. In addition, infection with EEE virus can lead to permanent neurologic sequelae. Horses and humans are considered dead-end hosts for EEE and WEE viruses, because they rarely develop viremia of sufficient titer to allow for further vector-borne transmission.

Emus (Dromaius novahollandiae) also develop disease following alphavirus infection. The EEE virus appears largely viscerotropic in emus, and infection is associated with a rapid onset of clinical signs of disease and high mortality rate. Clinical manifestations include disseminated intravascular coagulation, severe hemorrhagic enterocolitis, and blood-tinged vomitus. Infection with the WEE virus may cause various clinical abnormalities in emus, including anorexia, weight loss, an S-shaped curvature of the cervical portion of the spine, drowsiness, gait abnormalities, recumbency, paralysis, and death. The role that emus play in maintenance and transmission of EEE and WEE viruses remains unclear, but it is conceivable that they function as virus reservoirs rather than as dead-end hosts, thereby posing a health risk to humans and animals.

Vaccination of horses against alphaviruses is widely practiced and provides effective protection from infection. The susceptibility of emus to infection with EEE and WEE viruses has prompted the extralabel use of commercially available equine vaccines in emus. Anecdotal reports from regions of the country in which EEE and WEE virus infections are endemic suggest that this approach is efficacious, despite the lack of scientific evidence supporting such usage. The immunogenicity and efficacy of these commercially available equine vaccines in emus have only been cursorily examined, and to date, demonstrations of the immunogenicity and efficacy of these vaccines administered to emus under controlled conditions, including challenge exposure with virulent virus, have not been reported.

Therefore, the purposes of the study reported here were to evaluate the humoral immune response of emus vaccinated with commercially available equine...
polyvalent or experimental monovalent alphavirus vaccines and to determine whether vaccinated emus were protected against challenge with EEE virus. Viremia levels, shedding, and transmission of the EEE virus were also examined in unvaccinated emus.

Materials and Methods

Animals—Twenty-five 12- to 14-month-old unvaccinated emus were selected by convenience sampling from a group of approximately 150 yearling animals representing both sexes. Animals were housed for the immunogenicity phase of the study at their point of origin in Colorado in a single 100 x 200-ft open-air community pen with shelter available. A commercial grower-finisher ration was available ad libitum. Although infection with WEE virus has been documented in Colorado, such infections are rare; infection with EEE virus has not been reported from any state as far west as Colorado.

Vaccines and vaccination protocol—Four vaccine preparations were evaluated. Two were commercially available polyvalent vaccines labeled for immunization of horses; the other 2 were experimental monovalent vaccines intended for immunization of humans. Each of the vaccines was administered to emus, using the dosage recommended for its target species. Emus were identified by numbered leg bands and randomly assigned to 5 groups of 5 birds each. Group-1 birds received a commercially available polyvalent equine vaccine (inactivated EEE and WEE viruses and tetanus toxoid), group-2 birds received a commercially available polyvalent equine vaccine (inactivated EEE, WEE, and Venezuelan equine encephalomyelitis viruses), group-3 birds received an inactivated monovalent EEE virus vaccine, and group-4 birds received an inactivated monovalent WEE virus vaccine. Birds in group 5 were not vaccinated. The equine vaccines were administered by IM injection into the caudoproximal aspect of the right thigh on days 0 and 28, according to the vaccine manufacturers’ recommended schedule for horses. The inactivated monovalent EEE virus vaccine was administered SC (0.5 ml) in a similar location on days 0 and 28. The inactivated monovalent WEE virus vaccine was administered SC (0.5 ml) in a similar location on days 0, 7, and 28.

Evaluation of humoral immune responses—Blood samples were collected by means of jugular venipuncture from all birds in groups 1 through 4 on days 0 and 28. Serum samples were diluted 1:10 prior to analysis, and all samples were tested in duplicate. For the plaque reduction assay, serum samples were diluted 1:10 in BA-1 medium (medium 199 supplemented with 1% bovine serum albumin, 0.35 g of sodium bicarbonate/L, 100 U of penicillin/ml, 100 µg of streptomycin/ml, and 1 µg of amphotericin B/ml). Approximately 150 plaque-forming units (pfu) of EEE virus (NJ/60) or WEE virus (Fleming) were added to each test and virus control well. Plaque development on Vero cell monolayers was determined by overlaying with medium 199 supplemented with 1% agarose, 0.75% DEAE-dextran, 0.2% neutral red, and 1% fetal bovine serum. Mouse hyper-immune ascitic fluid against EEE or WEE virus was used as the positive control and was tested in parallel with the experimental samples. Plaques were recorded 3 days after overlay application, and the relative activity of antibody in each test serum sample was enumerated as the percentage neutralization of input virus. Differences in neutralizing antibody responses among groups and over time were tested by means of repeated measures ANOVA. \( P < 0.05 \) were considered significant.

Challenge exposure and virus isolation—Three of the 5 birds in groups 1 and 2 were given a booster vaccination on day 240, using the same vaccine given previously; the other 2 birds in each group did not receive booster vaccinations. All 10 birds in these 2 groups were subsequently challenged with EEE virus. Challenge exposure consisted of SC injection of approximately 2 x 10^7 pfu of EEE virus (NJ/60; multiple passages). Blood samples were collected on day 240, on the day of challenge exposure (approx day 279 in birds that received a booster vaccination and day 293 in birds that did not receive a booster vaccination), every 12 hours for the first 2 days after challenge exposure, and every 48 hours thereafter until 14 days after challenge exposure. Birds were euthanatized 14 days after challenge exposure; blood samples were collected at the time of euthanasia. Swab samples of feces and saliva were also obtained from each bird at the same times blood samples were obtained; swab samples were diluted in BA-1 medium.

Two unvaccinated control (group-5) birds were also challenged in a similar manner. Blood, feces, and saliva samples were collected into BA-1 medium at the time of challenge exposure and 12 and 24 hours later. Both birds had clinical signs of infection 24 hours after challenge exposure and were euthanatized 28 hours after exposure. A third unvaccinated control bird that was not challenge exposed was housed with the 2 challenge-exposed control birds to assess the possibility of direct virus transmission. Blood, feces, and saliva samples were collected at the time the challenge-exposed birds were inoculated, 12 and 24 hours later, and every 24 hours thereafter. Clinical signs of infection were apparent approximately 72 hours after the pen-mates had been inoculated with the virus, and the bird was euthanatized at this time. Blood, feces, and saliva samples were taken at this time.

Blood samples and samples of saliva, feces, and vomitus from the unvaccinated birds were assayed for virus. Samples were diluted serially in BA-1 for virus isolation and titration and inoculated onto Vero cell monolayers, as described for the plaque neutralization assays. Plaques were enumerated 3 days later, and titers were calculated.

Challenge exposure studies were carried out during the winter to preclude the possibility of vector transmission. All 13 birds were housed in a Biosafety Level 3 containment facility at Colorado State University and fed a commercial grower-finisher ration ad libitum. Handlers were appropriately vaccinated and clothed. Waste and tissues were decontaminated and incinerated on-site. Birds were monitored throughout the study for clinical manifestations of infection. Because of the difficulties and safety issues associated with handling these birds, body temperatures were not recorded.

Results

All 4 vaccines induced detectable neutralizing antibody titers (Tables 1, 2, and 3). Birds that received the 2 commercially available polyvalent equine vaccines developed generally comparable neutralizing antibody titers against EEE and WEE viruses. For all 4 vaccinated groups, antibody titers 7 days after primary immunization were significantly greater than preimmunization titers. The EEE virus neutralizing antibody titers for birds in group 1 were significantly higher than those for birds in group 2 on days 7 through 35 but not thereafter. Antibody titers to WEE virus were not significantly different between birds in groups 1, 2, and 3.
Although efficacy of the monovalent experimental vaccine was not examined, immunogenicity of these vaccines appeared comparable to that of the polyvalent vaccines.

None of the birds that received either of the polyvalent equine vaccines (groups 1 and 2) developed clinical signs of disease during the 2 weeks following challenge exposure with an otherwise lethal dose of EEE virus. In addition, none of the birds from these groups developed detectable viremia or shed virus in feces or oral secretions during that time. Protective immunity to challenge exposure approximately 9 months after the initial vaccination series was manifest regardless of whether the emus received a booster vaccination 7 months after the initial series.

Both unvaccinated control birds were clinically normal 12 hours after challenge but developed severe illness by 24 hours. Clinical signs of infection included listlessness, excessive yawning, prolonged periods of recumbency, hematemesis, and hematochezia. Virus was not detectable in blood or secretions 12 hours after challenge. By 24 hours after challenge, these birds had developed a viremia $>10^9$ pfu/ml of blood ($3 \times 10^9$ and $2 \times 10^9$) and shed virus in oral secretions and feces (mean, $10^9$ and $10^8$ pfu/ml). For humane reasons, both birds were euthanatized soon after clinical signs of disease appeared. The pen-mate that was not challenge exposed remained healthy for an additional 48 hours, at which time identical clinical signs were evident.

Viremia of $10^7$ pfu/ml in blood and $10^9$ pfu/ml in feces or oral secretions during that time. Protective immunity to challenge exposure approximately 9 months after the initial vaccination series was manifest regardless of whether the emus received a booster vaccination 7 months after the initial series.
regurgitated material (10³ pfu/ml) were detected. This bird was also euthanatized soon after the onset of clinical signs.

Discussion

Commercial production of emus for meat and other by-products began in the United States in the mid-1980s, and despite precipitous declines in marketability, large numbers of birds continue to be produced annually nationwide. The first confirmed outbreak of EEE virus infection in emus was reported in Louisiana in 1991, and in 1992, a similar outbreak of WEE virus infection occurred in Oklahoma. Every year since then, high morbidity and mortality rates caused by EEE and WEE virus infections have been reported for emu flocks in areas where vector populations support such infections.

Passerine birds serve as vertebrate amplifying hosts for EEE virus. Ornithophilic mosquitoes (Culiseta melanura) are the primary enzootic vector. Infection in passerine birds is typically subclinical. Although geographic variation exists, Aedes or Coquillettidia mosquitoes with less restrictive feeding habits serve as bridging vectors and move virus from infected birds into other avian species or mammals. The WEE virus is found predominantly in agroecosystems west of the Mississippi drainage and is maintained in a manner similar to EEE virus, with Culex tarsalis as its primary mosquito vector.

Because of their susceptibility to infection, emus may play a key role in the epidemiology of EEE virus in regions in which the infection is endemic, but their contribution as a virus-amplifying host is unclear. They may play a part in vector-borne and direct transmission of virus to other emus and mammals, including humans. Results of the present study clearly support the supposition that emus are capable of serving as virus-amplifying hosts for vector-borne transmission. The 2 unvaccinated emus developed viremia in excess of 10⁹ pfu/ml of blood following inoculation with approximately 10⁷ pfu of EEE virus. Mitchell et al calculated that 10⁶ pfu of EEE virus/ml of egg white was sufficient to infect 50% of feeding Aedes albopictus mosquitoes. Komar et al observed that 100% of C melanura became infected after engorging on bob-white with 10⁶ pfu of EEE virus/ml of blood. Results of these studies suggest that emus, with viremia levels well above the threshold for efficient infection of feeding mosquitoes, could act as EEE virus-amplifying hosts for enzootic and bridge vectors.

In the present study, we found that infected emus could not only shed virus into the environment but also transmit EEE virus to another emu in the absence of mosquito vectors, as has been previously demonstrated with turkeys and pheasants. Furthermore, the ensuing infection was as fulminant as in the birds receiving virus parenterally. It seems likely that such direct transmission occurs via the aerosol or fecal-oral route. Pathologic abnormalities in emus with EEE virus infection have been described, and virus has been identified in liver, spleen, and mucosal sections of the gastrointestinal tract. Although it has been suspected that infected birds shed virus into the environ-
view of the large amounts of EEE virus in bloody vomitus and feces from infected birds, it is surprising that no human cases of EEE virus infection have been associated with infected emus. Infected emus should be segregated from a flock to reduce the potential for direct transmission, and owners and veterinarians should take precautions to minimize their exposure to virus in excretions and secretions. In view of the results of this study, vaccination of emus in areas in which EEE virus infection is endemic appears justified to protect owners as well as birds from this serious disease.

References


