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## Induction of Neutralizing Antibodies in Reindeer (*Rangifer tarandus*) after Administration of a Killed West Nile Virus Vaccine

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**ABSTRACT:** In 2002, West Nile virus (WNV) infection with clinical neurologic disease and encephalomyelitis was described in reindeer (*Rangifer tarandus*). The susceptibility of reindeer to WNV prompted questions concerning vaccination of reindeer to prevent WNV infection. Between January and April 2003, eleven 2–4-yr-old, castrated male reindeer, some of which had antibody titers suggestive of prior exposure to WNV, were vaccinated three times at 4-wk intervals with a commercially available vaccine approved for use in horses. No adverse reactions to vaccination were noted. All vaccinated reindeer developed high neutralizing antibody titers to WNV, as determined by the plaque reduction neutralization test. Reindeer without antibody titers from previous natural exposure to WNV required a primary vaccination and one or two booster vaccinations for development of neutralizing antibody to WNV. Protective efficacy of vaccination was not evaluated. Vaccination of reindeer for WNV may be warranted in certain circumstances combined with management practices to limit exposure to potential vectors.

**Key words:** Flavivirus, *Rangifer tarandus*, reindeer, vaccination, West Nile virus.

Since the introduction of West Nile virus (WNV) to North America in 1999, the known range of susceptible host species has increased to include numerous species of wildlife, including various mammals and reptiles in which clinical disease had not previously been recognized (Buckweitz et al., 2003; Kiupel et al., 2003; Malakoff, 2003; Miller et al., 2003; Palmer et al., 2004). In the fall of 2002, WNV-induced neurologic disease with lymphohistiocytic encephalomyelitis was described in reindeer (*Rangifer tarandus*) in the Midwestern USA (Palmer et al., 2004). Recognition of the susceptibility of reindeer to WNV infection prompted questions concerning vaccination of reindeer to prevent

WNV infection. The purpose of this report is to describe virus-neutralizing antibody responses of reindeer after vaccination with a killed WNV vaccine.

In the spring of 2002, a group of reindeer ( $n=20$ ) were moved from southern Michigan, USA (41.9°N, 85°W), to Ames, Iowa, USA (42°03'N, 93°63'W), for research on reindeer immunology. Sera were collected from all reindeer shortly after arrival in Iowa. In the fall of 2002, three reindeer from this group developed severe neurologic disease from WNV-induced encephalomyelitis and were euthanized due to the poor prognosis (Palmer et al., 2004). Blood was collected and serum harvested from clinically affected reindeer as well as those not affected during this period. Nine of 17 (53%) remaining reindeer developed neutralizing antibody titers of  $\geq 1:100$ , as determined by the plaque-reduction neutralization test (PRNT) (Palmer et al., 2004).

Vaccination of 11 of the remaining reindeer was conducted during the winter of 2002–03. A commercially available equine vaccine WNV infection (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) was used according to manufacturer recommendations for vaccination of horses. Briefly, 1 ml was administered intramuscularly; three doses were given with a 4-wk interval between injections. Serum was collected for measurement of virus neutralizing antibody titers prior to the initial vaccination, prior to each booster, and 4 wk after the last booster vaccination.

Antibody titers to WNV were determined using PRNT as described (Ostlund et al., 2001). Briefly, serum dilutions of 1:

TABLE 1. Virus neutralizing antibody titers in reindeer prior to and after vaccination with a killed West Nile virus vaccine.

Animal identification	Days relative to vaccination (day 0) <sup>a</sup>					
	-230	-133	0	28	56	84
100	<1:10 (-) <sup>b</sup>	≥1:100 (+)	≥1:100 (+)	≥1:100 (+)	≥1:100 (+)	>1:100 (+)
102	<1:10 (-)	<1:10 (-)	<1:10 (-)	<1:10 (-)	1:100 (+)	>1:100 (+)
104	<1:10 (-)	<1:10 (-)	<1:10 (-)	<1:10 (-)	1:10 (+)	>1:100 (+)
106	<1:10 (-)	≥1:100 (+)	1:10 (+)	≥1:100 (+)	≥1:100 (+)	>1:100 (+)
107	<1:10 (-)	<1:10 (-)	<1:10 (-)	<1:10 (-)	1:10 (+)	1:100 (+)
111	<1:10 (-)	≥1:100 (+)	1:10 (+)	≥1:100 (+)	≥1:100 (+)	>1:100 (+)
112	<1:10 (-)	≥1:100 (+)	≥1:100 (+)	≥1:100 (+)	≥1:100 (+)	>1:100 (+)
114	<1:10 (-)	<1:10 (-)	<1:10 (-)	<1:10 (-)	1:10 (+)	1:100 (+)
115	<1:10 (-)	<1:10 (-)	<1:10 (-)	<1:10 (-)	1:10 (-)	1:100 (+)
116	<1:10 (-)	≥1:100 (+)	≥1:100 (+)	≥1:100 (+)	≥1:100 (+)	>1:100 (+)
117	<1:10 (-)	<1:10 (-)	<1:10 (-)	<1:10 (-)	1:10 (-)	1:100 (+)

<sup>a</sup> Reindeer were vaccinated on days 0, 28, and 56. Blood was collected prior to administration of vaccine at each time point.

<sup>b</sup> Data are expressed as antibody dilution with plaque reduction of 90% (+) or <90% (-). Natural exposure to West Nile virus occurred in this group of reindeer sometime between 230 and 133 days prior to vaccination.

10 and 1:100 were examined for virus-neutralizing antibody using 100 plaque-forming units of WNV (North American isolate, crow origin, 99-34940). Virus-serum mixtures were incubated at 37 C for 75 min and then added to flasks of confluent Vero cell monolayers. Flasks were incubated for 60 min at 37 C and then overlaid with agar and incubated for 72 hr. A second agar overlay containing neutral red was then added and flasks examined the following day. Plaque reduction of ≥90% was recorded as positive. A PRNT titer of ≥1:10 was considered significant. Serum that neutralized 100% of the virus was assumed to have a titer of ≥1:100.

Animals were monitored twice daily. No undesirable effects of vaccination were observed either in reindeer that had neutralizing-antibody titers to WNV before vaccination or in reindeer without neutralizing antibody titers before vaccination. Subjectively, no change in feed consumption was noted during the study period and no visible or palpable reactions at the vaccine injection sites were noted. As reindeer were housed outside during the study, the vaccination series was conducted through the winter and early spring (January to April) to minimize vector exposure.

Shortly after arrival in Iowa (230 days before vaccination), antibody titers to WNV were not detected in any of the reindeer, suggesting no prior exposure to WNV. At the time that clinical signs of WNV-induced encephalomyelitis were seen in three reindeer (133 days prior to vaccination), five of the 11 reindeer in this study had neutralizing-antibody titers of ≥1:100, while six of 11 had no evidence of antibody titers to WNV (Table 1). Between September 2002 (133 days prior to vaccination) and the day of the initial vaccination in January 2003 (day 0), none of the reindeer negative for WNV exposure had developed detectable antibody titers to WNV. However, a decrease in antibody titer was seen in two reindeer (#106, #111). In these two reindeer, a single vaccination on day 0 acted as a booster, resulting in an increase in titer when examined 4 wk later (day 28). Four of six reindeer that did not have antibody titers to WNV prior to vaccination developed neutralizing titers of ≥1:10 by day 56 after initial vaccination and one booster vaccination. However, two of six reindeer required initial vaccination and two booster vaccinations to induce neutralizing titers of ≥1:100 by day 84.

In the present study, neutralizing antibodies persisted for at least 133 days after

natural infection, although a decrease in titer was seen in two reindeer during this time. With no previous research on the humoral response of reindeer to WNV, it is not possible to know if reindeer that began the vaccination series as negative had already been exposed to WNV and titers had declined to undetectable levels prior to the study. Although lack of an anamnestic response in these negative reindeer led us to believe that there was a lack of previous exposure prior to vaccination, lack of anamnestic response may also be due to a marginally effective vaccine.

After natural infection of horses, neutralizing antibodies may persist for at least 15 mo, while persistent titers have been demonstrated for  $\geq 2$  yr in humans (Hayes, 1989; Ostlund et al., 2001). Persistence of such titers may mean that protection from reinfection may also persist; however, such titers may confuse diagnosis in areas where West Nile virus has become endemic. Therefore, evidence of virus-neutralizing antibodies in a single serum sample cannot be used for proof of current infection, but rather, a fourfold increase in titer in appropriately timed paired serum samples should be used (Ostlund et al., 2001).

A commercially available killed vaccine was used in the present study. In other veterinary applications, live attenuated and killed vaccines have been used to control WNV infection in geese where 75–100% protection was seen, depending on the vaccine and number of booster vaccinations given (Lustig et al., 2000; Malkinson et al., 2001). Additionally, DNA vaccines for WNV have demonstrated significant protective effects even after a single intramuscular injection in horses and crows (*Corvus brachyrhynchos*) (Davis et al., 2001; Turell et al., 2003). The use of improved vaccines or improved vaccine technology that results in neutralizing-antibody production and protection after a single vaccination in reindeer remains to be explored.

The current study was not conducted as a serologic study to determine end-point

titers after vaccination. Antibody titers determined by PRNT were done to demonstrate seroconversion following vaccination. Vaccination of reindeer with a commercially available killed WNV vaccine licensed for use in horses induces virus-neutralizing antibodies when administered to reindeer. Although reindeer in the present study were not challenged with virulent WNV to examine vaccine efficacy, induction of virus-neutralizing antibodies, as demonstrated using the PRNT, may mean that some protection would be provided. However, with limited information concerning WNV in reindeer, it is not clear that neutralizing titers in Vero cells are indicative of virus-neutralizing potential in vivo. However, combined with demonstration of susceptibility of reindeer to WNV, vaccination of reindeer may be warranted under certain circumstances combined with other management practices to limit exposure to potential vectors.

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