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Inactivated West Nile Virus (WNV) vaccine, Duvaxyn WNV, protects against a highly neuroinvasive lineage 2 WNV strain in mice

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ABSTRACT

Background: Lineage 2 West Nile Virus (WNV) is endemic to southern Africa and Madagascar, and has recently been associated with encephalitis outbreaks in humans and horses in South Africa, central Europe, Italy and Greece. Commercial vaccines have mostly been evaluated against WNV lineage 1 strains and their efficacy against lineage 2 strains rarely reported.
Methods: To evaluate protection of Duvaxyn WNV vaccine against lineage 2 strains associated with encephalitis in South Africa, mice were vaccinated twice intramuscularly three weeks apart, and challenged four weeks later with highly neuroinvasive lineage 1 strain NY385/99 or lineage 2 strain SPU93/01. Neutralising antibody titres were measured at the time of challenge and three weeks later. Immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) were conducted on brains of mice that succumbed during the trial, on controls and on vaccinated mice that survived.
Results: Serum neutralising antibodies in vaccinated mice were detected but low three weeks after primovaccination. Three weeks post-challenge, vaccinated mice had significantly higher serum neutralising antibody titres against both lineages than unvaccinated controls. After challenge, all vaccinated mice remained healthy but all unvaccinated mice demonstrated severe neurological signs with 75% mortality rate. WNV was not detected in brains of vaccinated mice whereas virus replicated in most unvaccinated mice challenged with either lineage. Gross and microscopic lesions were found only in unvaccinated mice challenged with both lineages.
Conclusion: Duvaxyn WNV vaccine provided complete protection against challenge with lineage 2 WNV and stimulated significant cross protective neutralising antibodies in mice against lineage 2.

1. Introduction

West Nile Virus (WNV) is a mosquito-borne flavivirus in the family *Flaviviridae*, widely distributed throughout Africa, the Middle East, Asia, parts of Europe, Australia, North and South America and the Caribbean. WNV mainly circulate between ornithophilic mosquitoes vectors and birds while humans and horses are incidental hosts. Severe disease including meningoencephalitis, encephalitis, polio-like flaccid paralysis, and death occur in <1% of humans infected with WNV. Approximately 20% of horses infected with WNV exhibit clinical signs of which up to 90% display neurological signs and 42% of these may be fatal [1]. Signs of infection in horses include ataxia, weakness, recumbency, muscle fasciculation and high case fatality rate [2–5]. In 2002 15,000 clinical cases of equine WNV infection were reported in the USA and neuroinvasive disease was frequently identified [6]. Equine cases decreased following the introduction of an inactivated WNV vaccine for horses and as the virus became endemic [3,6,7].

Isolates of WNV fall mainly into two major genetic lineages: lineage 1 found in North America, North Africa, Europe and Australia,

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Table 1
Primovaccination protocol: group allocations and treatments administered to BALB/c mice.

Group	Day 0	Day 21	Day 49 (challenge day 0)	Day 49–70 (observe clinical signs and mortalities)	Day 70 (21 days after challenge)
1	Vaccinate 10 mice	Boost 10 mice	Challenge SPU93/01 Harvest 1 mouse Neutralising Abs and histopathology baseline	Harvest brain, liver, spleen of fatalities	Bleed Harvest remaining mice
2	Vaccinate 10 mice	Boost 10 mice	Challenge NY385/99 Harvest 1 mouse Histopathology baseline	Harvest brain, liver, spleen of fatalities	Bleed Harvest remaining mice
3	Vaccinate 10 mice	Boost 10 mice	Sacrifice and bleed 5 mice Neutralising Abs Sacrifice other 5 mice just for organs		
4	PBS 10 mice	PBS 10 mice	Challenge SPU93/01 Harvest 1 mouse Neutralising Abs	Harvest brain, liver, spleen of fatalities	Bleed Harvest remaining mice
5	PBS 10 mice	PBS 10 mice	Challenge NY385/99 Harvest 1 mouse Neutralising Abs	Harvest brain, liver, spleen of fatalities	Bleed Harvest remaining mice
6	PBS 11 mice Harvest one mouse as Baseline control	PBS 10 mice	PBS	Harvest brain, liver, spleen. 1 sacrificed and bled 7 d after challenge.	Bleed Harvest remaining mice
Summary of procedures			VNTs vaccinated and unvaccinated controls VNT on 1 SPU93/01 and NY385 challenged	Unvaccinated control groups IHC RT-PCR	Vaccinated groups and challenged survivors VNTs; RT-PCR and IHC

and lineage 2, endemic in central and southern Africa and Madagascar [8]. In 2004 and 2005, a lineage 2 strain isolated from encephalitic raptors in southern Hungary suggested spread outside the known geographic range [9]. Lineage 2 was subsequently identified as the cause of disease in horses in 2008 and 2010 in Hungary [10–12]; in humans in Russia in 2007 [13,14]; in birds in Austria in 2008 [15]; in humans in Greece in 2011 [13,16–20]; in horses in Romania, 2010 [21]; and in a human, dove and *Culex pipiens* mosquitoes in Italy, 2011 [22,23].

West Nile Virus (WNV) is widely distributed in South Africa [24,25]. Initially postulated not to be neurovirulent in humans and horses [24,26], cases of non-fatal encephalitis and fatal hepatitis in humans, fatal cases in a dog and ostrich chicks [8,27] and encephalitis in horses [28] have subsequently been associated with WNV lineage 2 in South Africa. Certain lineage 2 strains are highly pathogenic and neuroinvasive in mice [29,30]. An 18-month study of equine neurological disease in South Africa identified WNV in 19% of clinical cases. WNV associated cases all presented with severe neurological disease and 85% were euthanised or died from encephalitis. Clinical signs resembled those of lineage 1 strains described in the USA. All cases positive by RT-PCR were confirmed as lineage 2 by DNA sequencing and phylogenetic analysis [28]. Continued surveillance of horses during the past 3 years suggests that WNV accounts for approximately 7–21% of equine neurological cases annually in South Africa with a 39% mortality rate (unpublished data). All equine cases in South Africa have been associated with lineage 2 apart from a single fatal lineage 1 case identified in 2010 [31]. A WNV vaccine is therefore warranted for horses in southern Africa.

Commercial WNV vaccines for horses were developed for use against lineage 1 strains in the USA. Vaccines licensed in the USA include a canary pox WNV recombinant vaccine (Recombitek rWNV, Merial Canada Inc.) [32], an inactivated WNV vaccine (Innovator®, Pfizer Animal Health) and a DNA vaccine [7]. All these vaccines contain lineage 1 WNV antigens. A canarypox-WNV recombinant vaccine was recently shown to protect against lineage 2 strains [32,33].

To investigate the efficacy of an inactivated WNV vaccine currently licensed in the USA and Europe against lineage 2 strains, we conducted a primo-vaccination cross-lineage vaccine trial in which mice were vaccinated twice with the formalin inactivated WNV vaccine, Duvaxyn WNV (Pfizer) and then challenged with a highly neuroinvasive lineage 2 strain isolated from an encephalitic human in South Africa [8]. Mice aged six to eight weeks were previously shown to be a good model for the study of WNV neurological disease and highly sensitive to WNV infection [30]. Cross neutralisation and protection in mice in combination with cross neutralisation in vaccinated horses will support prior data on the efficacy of this vaccine and provide justification for its licencing in regions where lineage 2 threatens horses.

2. Materials and methods

2.1. Study schedule and vaccination protocol

The experimental schedule and treatment groups are given in Table 1. Six groups of 6–8-week-old mice, each consisted of 10 randomly selected male BALB/c mice. Ten mice per group were shown to be statistically sufficient to allow evaluation of the response to vaccination [34,35]. On study days 0 and 21, three groups (groups 1–3) were primovaccinated intramuscularly with 0.1 ml of Duvaxyn WNV vaccine (Batch 387BYA01E) (Zoetis, Zaventem, Belgium), and three control groups (groups 4–6) were administered 0.1 ml phosphate buffered saline (PBS) intramuscularly.

On day 49, 4 weeks after completion of primovaccination on day 21, vaccine group 1 and its control counterpart (group 4) were challenged intraperitoneally with 0.1 ml inoculum containing 1×10^4 $TCID_{50}$ lineage 2 WNV strain SPU93/01 per ml, and vaccine group 2 and its control counterpart (group 5) were similarly challenged with lineage 1 WNV strain NY385/99. Mice in vaccine group 3 were sacrificed on day 49 post-vaccination to determine neutralising antibody response to vaccination. The control group (group 6) was administered 0.1 ml PBS intraperitoneally. After challenge, all mice were monitored for signs of disease for 21 days until day 70 post-vaccination (day 21 post-challenge). Any mouse that was suffering or exhibiting signs of severe disease was euthanised.

Blood samples were taken from one mouse in each of groups 1, 2, 4, 5 and 6 on day 49 post-vaccination (day 0 post-challenge)