West Nile virus serosurveillance in camelids

Michelle Anne Kutzler*

Oregon State University, College of Agricultural Sciences, 312 Withycombe Hall, Corvallis, Oregon 97331

Abstract

West Nile virus (WNV) encephalitis in camelids may result in clinical signs of disease ranging from anorexia, fever and facial tremors to incoordination, recumbency and death. Similar to humans and horses in which <1% of WNV infected individuals become severely ill, it appears that the majority of camelids infected by WNV are asymptomatic and recover uneventfully. Since camelids are reported to be at "low risk" for WNV infection, the objective was to determine the prevalence of subclinical WNV infection through a nationwide serosurveillance study. Jugular venous blood samples were collected from 322 camelids on 35 farms in 20 states. Owners provided information about each animal including species, gender, age, coat type (Huacaya or Suri), and coat color. End-point virus-neutralizing antibody titers were used to determine seropositive rate. Sixty-nine percent (18/26) of camelid farms from areas of known WNV activity had one or more seropositive animals. There was no effect of gender, age coat type or coat color on the seropositive rate. Alpacas produced a significantly higher titer compared to llamas following subclinical infection. However, significantly more llamas were seropositive compared to alpacas. Camelid owners should be vigilant in carrying out WNV preventative measures (e.g. vaccination, mosquito control). In addition, camelid owners should be aware of the sign of WNV related illness, since early treatment yields the best results.

Keywords: alpacas, encephalitis, llamas, viremia, West Nile virus

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***Corresponding author:** Michelle Anne Kutzler, DVM, PhD, DACT
Department of Animal and Rangeland Sciences, Oregon State University
Email: michelle.kutzler@oregonstate.edu; Phone: 541-737-1401; Fax: 541-737-4174; Mobile: 541-740-1434

Introduction

West Nile virus (WNV) was first discovered in 1937 in Africa and since then has spread to the Middle East, Europe, Asia and in 1999, North America. A flavivirus, WNV is transmitted by infected mosquitoes to birds, its natural host. Although uncommon, WNV may be transmitted to humans or other mammalian species, reptiles and amphibians by mosquitoes. While all mammalian species can become infected with WNV, surveillance programs within the United States have focused on infection rates in humans and horses.

In countries where WNV is enzootic, antibody seropositive rates in ruminant species approach 62%, although clinical signs of WNV infection in these species are infrequent (Olaleye OD et al 1990; Omilabu SA et al 1990). In the fall of 2003, more than a dozen camelids succumbed to WNV encephalitis and death throughout the Midwestern and Southwestern states. West Nile virus encephalitis in camelids results in anorexia, fever, facial tremors, incoordination, recumbency and death (Dunkel et al., 2004; Kutzler et al., 2004a; Yaeger et al., 2004).

Similar to humans and horses in which <1% of WNV infected individuals become severely ill, it appears that the majority of camelids infected by WNV are asymptomatic and recover uneventfully. However, alpacas may be more susceptible to WNV infection than other camelids (Kutzler et al., 2004a). Since camelids are reported to be at "low risk" for WNV infection (Ramsey et al., 2003), the objective was to determine the prevalence of subclinical WNV infection through a nation-wide serosurveillance study.

Methods

Jugular venous blood samples were collected from 322 camelids on 35 farms in 20 states (California, Colorado, Florida, Georgia, Idaho, Iowa, Louisiana, Maine, Maryland, Montana, New Jersey, New York, Ohio, Oregon, South Carolina, South Dakota, Texas, Vermont, Washington, Wisconsin) from January 2003-July 2005. Animals sampled for this study were older than one year of age, had lived on the farm of residence since birth or since 1999, had not been vaccinated against WNV and had never shown signs of WNV encephalitis. With the exception of 58 alpacas from nine farms in Idaho, Oregon and Washington, animals were selected from farms located in counties where WNV had been detected through surveillance methods (mosquito pools, serosurveillance in chickens or horses, clinical disease in

humans or horses). Owners were requested to provide information about each animal including species, gender, age, coat type (Huacaya or Suri), and coat color.

Serum was separated from the blood samples aseptically following centrifugation. End-point virus-neutralizing antibody titers against WNV were determined as previously described by Kutzler et al., 2004b). Briefly, serum samples were heat-inactivated for 30 minutes at 56ºC. Two-fold serial dilutions (1:2 to 1:1024) of each serum sample were tested in duplicate on 96-well microtitration plates. An equal volume of diluent containing 10^2 $TCID_{50}$ of WNV and 3% guinea pig complement was added to each well. Each serum-virus-complement mixture was incubated at 37ºC for 60 minutes and then an equal volume of a WNV-susceptible cell suspension (Vero cells) was added to each well. Wells were incubated at 37ºC (5% CO_2) for 6 days and individually examined with an inverted microscope for evidence of virus replication (cytopathogenic effect). The virus-neutralizing antibody titer of each serum sample was determined as the inverse of the highest dilution that inhibited the cytopathogenic effect of the test virus. The geometric mean titer (GMT) was determined for each individual duplicate sample. A GMT < 1:8 was defined as a seronegative response. Seropositive rate was compared with species and coat type (alpaca only) using a *z* test for difference of proportions; and with gender, coat color and age using a Chi-square test. Serum neutralizing WNV titers were compared with species using a Student's *t* test with unpooled variance, with gender using a Chi-square test, with coat type using an unpooled difference of means, with coat color using a one-way ANOVA; and with age using linear regression. Microsoft Excel and Graph Pad Prism® software were used. Significance was defined as $p<0.05$.

Results

Sixty-nine percent (18/26) of camelid farms from areas of known WNV activity had one or more seropositive animals. None (0/9) of the camelid farms from areas with no WNV activity had seropositive animals. Of the animals surveyed from farms in WNV endemic areas, 29% (76/264) were seropositive. The seropositive rate was ≥70% of the animals tested from six farms located in Colorado (n=3), Maryland, South Dakota and Texas. For seropositive camelids, WNV serum neutralizing titers ranged from 1:8-1:1448. The GMT of WNV serum neutralizing

antibodies was 1:281 for seropositive camelids.

Samples were received from 278 alpacas, 42 llamas and 2 camels (one Bactrian and one Dromedary). Significantly more llamas were seropositive compared to alpacas (p=0.025; Table 1). However, alpacas produced a significantly higher titer compared to llamas following subclinical infection (p<0.001). Neither of the camels (0/2) were seropositive.

Table 1. Seropositive rate in the four camelid species surveyed from areas with WNV activity. Significantly more llamas were seropositive compared to alpacas (*p<0.05).

Species	***n***	**% seropositive**
Alpaca	220	28
Llama	42	45*
Bactrian	1	0
Dromedary	1	0

Samples were received from 190 females, 121 intact males, and 7 castrated males. Gender was specified in all but four animals. There was no effect of gender on seropositive rate (females compared to all males, p=0.71; females compared to intact males, p=0.65; Table 2) or titer (p=0.38). In addition, age did not influence the seropositive rate (p=0.60; Table 3) or titer (R=0.058, R^2=0.003).

Table 2. Gender did not influence the seropositive rate in camelids. [a]Number of camelids surveyed in areas with WNV activity. Gender was not provided for 4 alpacas.

Gender	***n*[a]**	**% seropositive**
Female	171	29
Intact Male	82	35
Gelded Male	7	0

Table 3. Age did not influence seropositive rate. *Number of camelids surveyed in areas with WNV activity. Age was not provided for 46 camelids.

Age (years)	***n****	**% seropositive**
1	66	24
2	28	19
3	22	36
4	10	20
5	15	20
6	17	6
7	16	37
8	10	20
9	13	15
11	5	20
12	5	20
14	2	100
15	3	33

Samples were received from 232 Huacaya alpacas and 46 Suri alpacas. Coat type was specified in 86% (278 of 322) of samples. There was no effect of coat type on the seropositive rate (p=0.19) or titer (p=0.08) of animals from farms in WNV endemic areas (Table 4). Coat color was specified in 86% (276 of 322) of samples, with 14 different colors being reported including black, white, beige and multiple shades of brown, gray and fawn. For purposes of comparison, coat color was categorized as light (n=74), medium (n=101), dark (n=76) and contrasting (n=25) (Table 5). There was no influence of coat color on seropositive rate (p=0.28) or titer (p=0.71).

Table 4. There was no influence of coat type on seropositive rate in the alpacas surveyed from WNV endemic areas.

Coat Type	***n***	**% seropositive**
Huacaya	187	26
Suri	32	38

Table 5. Coat color did not influence seropositive rate. [a]Number of camelids surveyed in areas with WNV activity. Coat color was not provided for 60 alpacas.

Coat Color Group	**Colors Included In Each Group**	***n*[a]**	**% seropositive**
Light	White	59	27
Medium	Beige, Fawn, Light Gray, Light Brown	79	15
Dark	Black, Dark Brown, Medium Brown, Bay Black, Dark Silver	55	25
Contrasting	White with Brown and/or Black	25	28

Discussion

West Nile virus encephalitis in camelids can result in clinical signs of disease ranging from anorexia, fever and facial tremors to incoordination, recumbency and death. Similar to humans and horses in which <1% of WNV infected individuals become severely ill, it appears that the majority of camelids infected by WNV are asymptomatic and recover uneventfully. A larger proportion of the llamas in this study were seropositive compared to the alpacas. Since personal correspondence and a number of articles suggest a disproportionate amount of symptomatic WNV infection in alpacas instead of other camelids (Table 6) (Dunkel et al., 2004; Kutzler et al., 2004a; Yaeger et al., 2004), this suggests that alpacas may be more susceptible to WNV related illness while llamas are more likely to seroconvert without developing symptoms.

Table 6. Summary of 31 confirmed WNV encephalitis infections in camelids during the study period.

State	# of Cases & Species	Year
Arizona	1 Alpaca	2003
Colorado	8 Alpacas	2003
Georgia	1 Llama, 1 Alpaca	2003, 2005
Idaho	1 Alpaca	2004
Illinois	1 Llama, 1 Alpaca	2004
Iowa	2 Alpacas	2003, 2004
Minnesota	1 Alpaca	2003
New Mexico	5 Llamas, 2 Alpacas	2003
New Jersey	1 Alpaca	2004
New York	1 Alpaca	2004
North Carolina	1 Alpaca	2006
South Carolina	1 Alpaca	2005
Wyoming	2 Llamas, 1 Alpaca	2003

With only two camels involved in the study, it is impossible to draw any conclusions about the susceptibility of Old World camelids. However, studies in North Africa have reported WNV seropositive rates in Dromedary camels ranging from 5.2% (Vasil'ev et al., 2005) to 13% (Touil et al, 2012) to 29% (El-Harrak et al., 2011), indicating a similar seroprevalence as seen in llamas and alpacas in WNV endemic areas of North America. The author was not able to find a study describing a species (Dromedary versus Bactrian), gender, or age on WNV seroprevalence in camels, nor a study describing clinical WNV encephalitis in camels.

Gender does not appear to be a risk factor for WNV infection. Suri alpacas had a greater rate of seropositivity compare to their Huacaya counterparts, but with so few Suri alpaca samples received more research would have to be done to establish a statistically significant correlation. Coat color statistics were taken because there is discussion among camelid owners about whether mosquitoes demonstrate a color preference when it comes to feeding on camelids. Particularly there is concern that certain mosquito species' attraction to dark colors (Wen et al., 1997) may apply to which camelids they feed on. However, our results did not show a strong correlation between seropositivity rate and coat color. The results suggest that breeders and owners of camelids, especially if they have particularly valuable herd sires or breeding stock, need to be extra vigilant in carrying out preventative measures (e.g. vaccination, mosquito control). In addition, camelid owners and breeders should be aware of the signs of WNV related illness, since early treatment yields the best results (Kutzler et al., 2004a).

Conclusions

Educating camelid owners about the higher prevalence of subclinical WNV infection in llamas compared to alpacas is important. By taking this information into account, better decisions can be made regarding the value of preventing WNV by vaccination to protect the health and productivity of their herd.

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