A serological survey on antibodies against West Nile virus in horses of Khuzestan province

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Abstract:

BACKGROUND: West Nile virus (WNV) is a vector-borne agent that is maintained within a bird-mosquito cycle. In humans and equids, infection by this agent is usually asymptomatic, or characterized by a mild febrile illness. However, fatal meningo-encephalitis or encephalitis may occur. **OBJECTIVES:** The aim of this study was to evaluate the prevalence of WNV infection and correlation of this organism with host and environmental determinants in horses in Khuzestan province. **METHODS:** In 2011-2012, serum samples of 155 horses were randomly collected from 7 zones of Khuzestan province and were examined by ELISA assay. **RESULTS:** Seroprevalence of WNV infection was 70.3% (95% CI: 63.1-77.5%). Statistical analysis showed that age, zone, presence of lake, type of bed, time of sampling, staying out of the stable after sunset and the method of insect control are significantly associated with infection ($p<0.05$) but sex, presence of river, wall condition, presence of rubbish dump and history of disease are not significantly associated with infection ($p>0.05$). **CONCLUSIONS:** The results of the present study confirm that the WNV infection exists in Khuzestan province. Considering the local weather conditions and the facility of vector-borne transmission, the health authorities should take measures to prevent and control the infection.

Introduction

West Nile (WN) fever and WN encephalitis are serious diseases in both humans and horses. The causative agent is the West Nile virus (WNV; family Flaviviridae, genus Flavivirus) (Mukhopadhyay et al., 2003; Brinton et al., 2009). WNV is transmitted by mosquitoes. Culex pipiens serve as the primary enzootic vector (Hullinger, 2009) and passerine birds serve as the amplification host (Vander Meulen et al., 2005).

WNV infects a wide range of vertebrates (Smith and George, 2009). Human and Equidae are sensitive to infection (Kinpe et al., 2001). This disease was first reported in Africa in 1937(Smithburn et al., 1940), followed by reports in the Middle East, West Asia and Europe in the 1950s and 1960s (Castillo-Olivares and Wood, 2004). The rapid expansion of WNV in the Western hemisphere, following the invasion of this virus into the USA in 1999 (Asnis et al., 2000).WNV infections have never been reported in Iran but serological investigations in the 1970s indicated 10% and 26.6% antibody prevalence rate in human population in several provinces (Naficy and Saidi, 1970; Saidi, 1974; Saidi et al., 1976). Recent studies by real-time RT-PCR and ELISA methods on blood donors revealed negative results for viral RNA

detection; however, 5% serological positive results were reported (Sharifi et al., 2010). From 2003 to 2007, a molecular and serological study was carried out to determine the WNV status in different species of wild water birds in Iran. Samples were collected from 519 birds representing 26 different species. Out of 519 serum samples tested for WNV antibodies by virus neutralization and immunofluorescence methods, 78 samples (15%) were positive (Fereidouni et al., 2011). Ahmadnejad et al. conducted the first large-scale serosurvey for detection of West Nile virus in the equine population in Iran. Blood samples were obtained during 2008-2009 from 1054 equines from 260 districts in 27 provinces. The overall seroprevalence rate for WNV neutralizing antibodies was 23.7 % (Ahmadnejad et al., 2011).

WNV infections in Equidae (horses, donkeys or mules) are comparable to human infections. As in humans, clinical signs are only seen in a small percentage of cases of infection in Equidae; however, 10% of them develop a neurological disease. The severity of clinical signs is influenced by many factors such as age, method of rearing, housing conditions, virulence of the strain, genetic susceptibility and prior infection with other flaviviruses (Kinpe et al., 2001). Mild cases were defined as equids exhibiting lethargy, decreased appetite, muscle twitches and mild lameness; nonetheless, they required minimal nursing care. Moderate cases were defined as equids with wobbly gait, difficulty eating, signs of colic, reluctance to move, hyper sensitivity to noise and touch, altered awareness; they need more intensive veterinary management. The most common neurological signs of severe cases were equids that were recumbent, unable to rise and had seizures. These equids required extensive and continued nursing care during their illness (Hullinger, 2009). The reported mortality rates among clinically affected horses stretch from 20 to 57%in the USA, France and Italy (Steinman et al., 2002; Ward et al., 2004; Murgue et al., 2001)

Since equines are often spatially dispersed within human populations_ and outbreaks in equine populations generally result in high case fatality rates_ reports of equine cases might potentially provide early warning of corresponding WNV transmission in human populations. Therefore, assessment of virus presence in equine population can help us to determine risk of human infections.

In the present study, seroprevalence of WNV in Khuzestan (southwest of Iran) horses was assessed.

Materials and Methods

In 2011-2012, a serological study was carried out on horses in Khuzestan province to assess prevalence of West Nile virus antibodies and determine association between individual, environmental and herd management factors with the infection. In order to, serum samples from 155 horses were randomly collected from 7 zones in Khuzestan province (Ahvaz, Dezful, Shush, Ramhormoz, Abadan, Khoramshahr, and Baqmalek). The study unit was the individual and a questionnaire was used to collect information on 12 explanatory variables related to the individual, the environment and the site management practices (Table 1). Serum samples were screened for antibodies against WNV with a commercial competitive ELISA test (ID Screen West Nile Competition; ID vet, France) in accord with the manufacturer's instructions. For each sample, S/N percentage was calculated:

S/N=ODsample/OD negativecontrol×100

Samples with the S/N:

-Less than or equal to 40% were considered positive.

-Less than or equal to 50% and greater than 40% were considered doubtful.

-Less than 50% were considered negative.

Statistical analyses were performed by SPSS software version 16.0 (Statistical Package for Social Sciences, Chicago, IL, USA). Analysis of data was performed by Chi square test, Fisher exact test, and Logistic regression.

Results

Out of 155 sampled horses, 119 had antibodies against West Nile virus. Seroprevalence of WNV infection was 70.3% (95% CI: 63.1-77.5%). Seven variables out of the 12 studied variables were significantly associated with the serological status of horses ($p<0.05$). Among these 7 variables, 3 variables were related to the environment, one to the individual and 3 to practices on the site. Statistical analysis showed that the age, zone, presence of lake, type of

Table 1. Explanatory variables for association with seropositivity for WNV in horses of Khuzestan province.

Type	Variable	Definition
Individual		
	Age	4 categories (>6 months, 0.5-3, 3-10 and >10 years)
	Sex	Male/female
	History of illness	Yes/No
Related to the site		
	Outside after sunset	Animals that were not in stables after sunset
	Mosquito control	Treatment against mosquitoes
	Wall Condition	Blocks or bricks with coat
	Type of bed	soil, sawdust, cement, cement and lime, sand and lime and sand
Environmental		
	River	Presence of river or permanent water stream within a 0.5 km radius
	Ravines(lake)	Presence of ravines, generally dry but can be filled with water during the rainy season
	Proximity of rubbish dump	Presence of rubbish dump, water purification plant within a 1.5 km radius
	Zone	Ahvaz, Dezful, Shush, Ramhormoz, Abadan, Khoramshahr, and Baqmalek
	Time of sample collection	April, May, November, December and March

bed, type of wall, time of sample collection, staying out of the stable after sunset and the method of insect control was significantly associated with the infection ($p<0.05$). However, sex, presence of river, wall condition, presence of rubbish dump and history of disease was not significantly associated with the infection ($p>0.05$). Univariate logistic regression showed that presence of lake and staying out of the stable after sunset were risk factors for infection. The odds of infection also increased with the age of horses. Zone, time of sampling, method of mosquito control and the type of bed were strongly associated with the odds of the infection (Table 2).

Discussion

The horse populations were estimated to be about 1500 heads in Khuzestan province where were evaluated 10% of them for antibodies against WNV. WNV is maintained in nature through a transmission cycle between vector mosquitoes and reservoir birds (Komar, 2005; Balenghien, 2006; Jourdain, 2007).

Khuzestan province has more favorable conditions for propagation and circulation of the virus than the other provinces in Iran. This province has the most important wetlands, which are host to flora and fauna.

Studies dealing with WNV mainly report serological evidence of WNV transmission in resident and migratory birds and in equids without reference to the ecological characteristics of the putative WNV circulation areas (Dupuis et al., 2003, 2005; Estrada-Franco et al., 2003; Komar et al., 2003; Farfan-Ale et al.,2004; Komar and Clark, 2006; Pupo et al., 2006). WNV infections have never been reported in Iran and serological investigations in the 1970s indicated a high seroprevalence among the human populations in several provinces. The prevalence of WNV antibody in 1969 was 28.4% in residents of Khorasan and Khuzestan provinces (Naficy and Saidi, 1970; Saidi, 1974; Saidi et al., 1976). Recent studies by real-time RT-PCR and ELISA methods on blood donors revealed negative results for viral RNA detection; however, 5% had serological positive results (Sharifi et al., 2010). From 2003 to 2007, a molecular and serological study was carried out to determine the West Nile virus status in different species of wild water birds. Samples were collected from 519 birds representing 26 different species in Iran. Out of 519 serum samples tested for WNV antibodies, 78 (15%) were positive when tested using virus neutralization and immunofluorescence (Fereidouni et al., 2011). Ahmadnejad et al. conducted the first large-scale serosurvey of West Nile virus in the equine population in Iran. Blood samples were obtained from 2008 to 2009 from 1054 equines from 260 districts in 27 provinces. The overall seroprevalence rate of WNV neutralizing antibodies was 23.7%, which varied widely among the geographic location. The southern and western parts of the country were the most affected areas. Nine provinces had a prevalence of >30%, five provinces had a prevalence of >50%, and two provinces had a prevalence of >80% (Ahmadnejad et al., 2011).

Occurrence of vector borne disease results from

Table 2. Prevalence and odds ratio of WNV infection in horses of Khuzestan province.

	Prevalence	Odds ratio	95% CI for OR	P-value
Age				
<6 months	42.90	-	-	-
0.5-3 years	62.80	2.25	0.66-7.67	0.20
3-10 years	73.80	3.77	1.14-12.43	0.03
>10 years	84.80	7.47	1.8-30.99	0.006
Sex				
female	68.50	-	-	-
male	75.00	1.38	0.63-3.05	.042
History of illness				
No	69.10	-	-	-
Yes	81.90	1.94	0.53-7.16	0.32
Time of sampling				
Apr	55.60	-	-	-
May	90.90	8.00	0.94-67.86	0.08
Nov	57.10	1.07	0.32-3.58	0.90
Des	85.70	5.60	1.98-15.81	0.001
Mar	64.90	1.48	0.60-3.62	0.5
Zone				
Shush	50.00	-	-	-
Dezful	60.90	1.56	0.48-5.08	0.46
Khoramshahr	57.10	1.33	0.35-5.14	0.68
Ramhormoz	75.00	3.00	0.74-12.25	0.13
Baqmalek	95.70	22.00	2.51-192.93	0.005
Ahvaz	73.80	2.80	1.01-7.79	0.048
Proximity of Rubbish Dump				
Yes	68.20	-	-	-
No	72.90	1.25	0.62-2.51	0.53
River				
Yes	69.40	-	-	-
No	71.90	1.13	0.55-2.32	0.74
Ravines				
No	50.9	-	-	
Yes	81.6	4.29	2.07-8.89	<0.001
Type of bed				
Sawdust	16.70	-	-	
cement	71.40	12.50	2.46-63.49	0.002
Cement & lime	25.00	1.67	0.23-12.35	0.62
Soil	84.50	27.22	5.09-145.53	<0.001
Sand	88.90	40.00	3.05-524.83	0.005
Sand & lime	87.50	35.00	2.63-465.37	0.007
Wall Condition				
Block or brick without cover	53.80	-	-	-
Block or brick with cover	73.90	2.43	1.01-5.83	0.047
Fence	71.40	2.14	0.53-8.63	0.28
Mosquito control				
Chemical	53.80	-	-	-
Physical	66.70	1.71	0.61-4.80	0.31
Chemical & Physical	85.20	4.93	1.53-15.86	.007
Without control	88.10	6.34	2.21-18.19	.001
Outside after sunset				
No	51.00	-	-	-
Yes	72.90	3.67	1.77-7.61	<0.001

complex interactions, which makes the interpretation of risk factors models somewhat difficult. Risk factor may indicate the presence of reservoirs, host or vectors. The resylts of our study revealed that sero-prevalence of WNV infection was 70.3% (95% CI: 63.1-77.5%). The study found that 7 variables, out of the 12 initially selected, were strongly associated with infection. Three were environmental variables, 3 were linked to practices in the site and one was related to the host variable. This highlights the importance of practices in the site and environmental features. In this study, it was found that wetlands _northern areas of Khuzestan province (Baqmalek and Ramhormoz)_and presence of ravines were more likely suitable for WNV circulation compared to other areas. Wetlands were also a major component of the landscape associated with WNV cases in horses in the Camargue area-Rhoˆne River Delta in the South of France-particularly wet "sansou?¨re" and open water (Leblond et al., 2007) and in Texas (Ward et al., 2009). Whereas the number of lakes in Texas and the proximity to ponds in the Camargue proved to be a major risk indicator (Leblond et al., 2005; Ward et al., 2009), they were not associated with risk in North Dakota (Mongoh et al., 2007) or in Guadeloupe (paradel et al., 2009).

The increase in seroprevalence rate with equine age suggests recurrent circulation of WNV, as described in some serosurveys performed in endemic areas (Chevalier et al., 2006; Cohen et al., 1999).

In our study, horses kept outside after dusk and possibly exposed to a greater number of infective mosquitoe bites, had a significantly increased risk of being seropositive compared to those kept inside stables after dusk. Although the main WNV vector species in Khuzestan is unknown, this result may suggest that vector is rather exophilous (i.e. not entering buildings) and bites around dusk or at night, as do Culex species.

The presence of Culex pipiens, Culex modestus, and other important mosquito vectors for WNV (Azari & Hamidian, 2007) in Iran could also contribute to the transmission of the virus among different vertebrate hosts. The observed association between use of insect protection and exposure to WNV status was statistically significant. Species of mosquito implicated in WNV circulation can be targeted with insecticides either at their larval or adult stages. In this study, the chemical method tends to be more effective than the mechanical method.

In the present study, infection rates in different months were significantly different than the highest and lowest WNV antibodies titer observed in May and April respectively. Khuzestan is located at the cross roads of bird migratory routes, with birds coming from WNV-endemic regions and we know that any increase in temperature is likely to favor mosquito populations (Reisen, 2010). With this in mind, the high proportion of positive samples in May is explained. Drier summers and wetter winters may prevent mosquito summer population growth through a shortage of larval habitats at the critical times of the year; however, drought elsewhere causes a concentration of both mosquitoes and vertebrates around persistent water bodies which could promote transmission even under average, dry conditions (Monath,1980; DeGroote, et al., 2008; Brault, 2009). Under this scenario, disease hot spots could occur, even with relatively low average numbers of mosquitoes. An understanding of factors that increase equine WNV infection risk can be used to design and target disease control programs. Because WNV is a zoonotic agent, prevention and control programs such as vaccination and insecticides should be used in order to minimize the risk of infection in Khuzestan province.

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بررسی سرولوژیک حضور آنتی بادی علیه ویروس نیل غربی در اسبان استان خوزستان

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چکیده

زمینه مطالعه: ویروس نیل غربی از عوامل عفونی منتقل شونده توسط ناقل است که در چرخه انتقالی موجود میان پرندگان و حشرات حفظ می‌شود. بیماری ایجادشده توسط این عامل در انسان و اسب‌سانان معمولاً بدون علامت و یا به صورت بیماری با تب خفیف می‌باشد. با این حال مننگوآنسفالیت و یاآنسفالیت کشنده نیز ممکن است دیده شود. **هدف:** هدف از این مطالعه تعیین حضور سرمی ویروس نیل غربی و همچنین ارتباط این عامل با تعیین کننده‌های محیطی و میزبانی موجود در اسبان استان خوزستان می‌باشد. **روش کار:** طی سال‌های ۹۰-۱۳۸۹ نمونه‌های سرمی به صورت تصادفی از ۱۵۵ رأس اسب از ۷ بخش از استان خوزستان جمع‌آوری گردید و بوسیله روش الایزا مورد ارزیابی قرارگرفت. داده‌های جمع‌آوری شده با استفاده از آزمون مربع کای، آزمون دقیق فیشر و رگرسیون لاجستیک تحلیل گردیدند. **نتایج:** حضور سرمی آلودگی ۷۰/۳٪ (فاصله اطمینان ۹۵٪، ۷۷/۵-۶۳/۱) بود. بررسی آماری نشان دهنده این مطلب بود که سن، منطقه، وجود آبگیر، زمان نمونه گیری، نوع بستر، بیرون ماندن از اصطبل بعد از تاریکی هوا و روش کنترل حشرات به طور معنی‌داری با آلودگی به این ویروس مرتبط است. اما جنس، وجود رودخانه، نوع دیوار، وجود زباله دانی و دارا بودن سابقه ابتلاء به بیماریهای دیگر ارتباط آماری معنی‌داری با آلودگی به این ویروس نداشتند. **نتیجه‌گیری نهایی:** مطالعه حاضر نشان دهنده حضور ویروس نیل غربی در استان خوزستان می‌باشد. با توجه به وضعیت آب و هوایی منطقه و سهولت انتقال بیماری‌های ایجادشده بواسطه ناقلین، مد نظر قراردادن اقدامات کنترلی و پیشگیرانه توسط مسئولین الزامی می‌باشد.

واژه‌های کلیدی: اپیدمیولوژی، ویروس نیل غربی، اسب

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