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West Nile Virus: Seroprevalence in Animals in Palestine and Israel

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Abstract

West Nile virus (WNV) epidemiological situation in Israel and Palestine, due to their unique location, draws attention following to the global spread of West Nile fever (WNF). Although much information is available from Israel on clinical cases and prevalence of WNV, clinical cases are rarely reported in Palestine, and prevalence is not known. The objectives of this study were to determine WNV seroprevalence in various domestic animals in Palestine and to reevaluate current seroprevalence, force of infection, and risk factors for WNV exposure in horses in Israel. Sera samples were collected from 717 animals from Palestine and Israel (460 horses, 124 donkeys, 3 mules, 50 goats, 45 sheep, and 35 camels). Two hundred and ten horses were sampled twice. The level of WNV antibodies was determined using commercial Enzyme-linked Immunosorbent Assay (ELISA) Kit. Seroprevalence in equids was 73%. Seroprevalence in Israel (84.6%) was significantly higher than in Palestine (48.6%). Seroprevalence in horses (82.6%) was significantly higher than in donkeys and mules (39.3%). Multivariable statistical analysis showed that geographical area, landscape features (altitude), environmental factors (land surface temperature during the day [LSTD]), species, and age significantly influenced WNV seroprevalence. Fourteen of 95 (14.7%) sheep and goats and 14/35 camels (40%) sampled in Palestine were seropositive for WNV. Of the horses that were sampled twice, 82.8% were seropositive for WNV at the first sampling, and all remained seropositive. Three of the seronegative horses, all from Palestine, converted to positive when resampled (8.5%). The results indicate that domestic animals in Palestine were infected with WNV in the past, and the seroconversion indicates that WNV was circulating in Palestine in the summer of 2014. Control measures to prevent human infection should be implemented in Palestine. Anti WNV antibodies in domestic animals suggest that those species can be used as sentinels for WNV activity in areas where most horses are either seropositive or vaccinated.

Keywords: horse, Israel, Palestine, serology, West Nile virus

Introduction

West Nile virus (WNV), a member of the *Flavivirus* genus in the *Flaviviridae* family, is a mosquito-borne zoonotic arbovirus (Beck et al. 2013). WNV infection was first reported in the Mediterranean basin, in both Egypt and Israel, in the early 1950s, and neurological cases in humans were reported in Israel in 1957 (Spigland et al. 1958). During the summer of 2000, Israel experienced its largest recorded West Nile fever (WNF) outbreak that affected hundreds of people (Hindiyeh et al. 2001), and dozens of horses (Steinman et al. 2002). In the mid-1990s, WNV outbreaks occurred in Europe in countries where the virus had not been reported before, and in the summer of 1999 the virus crossed the Atlantic and reached the western hemisphere where cases were first reported in New York and spread in most of the United States of America and in neighboring countries in a 3-year interval (Chancey et al. 2015).

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Israel, due to its unique location in the crossroad for bird migration between Europe and Africa, draws attention during the global spread of WNF. The New York strain responsible for the outbreak in 1999 was most closely related to a strain isolated from the brain of a dead goose in Israel in 1998 (Malkinson et al. 1998) and had probably originated in the Middle East (Lanciotti et al. 1999). WNV appears to be expanding its geographical range in Europe, and large human epidemics occurred in the Balkan area during the last years (Sambri et al. 2013). Although much information is available from Israel, data regarding the prevalence of WNV in neighboring countries are limited. In Mashhad, Iran, 20 of 182 participants (11%) were positive for WNV immunoglobulin G (IgG) antibody in 2011–2012 (Meshkat et al. 2015). In Turkey, in samples that were collected in Mersin province in 2011, 32 of 266 (12%) humans were seropositive to WNV (Ergunay et al. 2014). According to the European Center for Disease Prevention and Control that report on WNV cases in the European region and the Mediterranean basin, in the last few years (2011–2016), 447 cases were reported from Israel, 1 from Palestine (in 2015), 2 from Syria (in 2016), and 1 in Egypt (in 2016) (http://ecdc.europa.eu/en/healthtopics/west_nile_fever/pages/index.aspx last accessed in February 2017).

Regarding WNV in animals, recent information is only available from Jordan, where one quarter of tested horses was seropositive (Abutarbush and Al-Majali 2014). According to the Office International des Epizooties/The World Organization for Animal Health (OIE) World Animal Health Information System, between the years 2005 and 2016 the disease was not reported in animals in Egypt, Lebanon, Syria, Saudi Arabia, Iraq, Jordan, or Palestine (www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasetimelines last accessed in April 2016). In contrast, between the years 2000 and 2012, nearly 1400 cases were reported in people in Israel (Anis et al. 2014), and dozens of cases were reported in horses by the Kimron Veterinary Institute (KVI) in their annual reports (32 in 2009; 13 in 2010; 8 in 2011; and 21 in 2012) (www.vetserv.moag.gov.il/Vet/all_Publications/dochotshnatiim/default.htm last accessed in April 2016).

Palestine is located on the eastern border of Israel and on the western border of Jordan. The objectives of this study were to determine, for the first time, WNV seroprevalence in various domestic animals in Palestine, including horses and donkeys, and to reevaluate current seroprevalence, force of infection, and risk factors for WNV exposure in horses in Israel. Furthermore, we wished to determine the seroprevalence in donkeys in Israel that was not determined before.

Materials and Methods

Study animals and design

Seven hundred and seventeen animals were sampled from Palestine and Israel (460 horses, 124 donkeys, 3 mules, 50 goats, 45 sheep, and 35 camels). The Israeli horse population is small (25,000–35,000 horses), and the Palestinian horse population is even smaller. Horses in Israel are used mainly for beauty show (Arabians) and for sport (English and Western riding); horses are also used for pleasure riding. In Palestine many of the horses are used for beauty show (Arabians).

Four hundred and sixty horses were sampled (353 from Israel and 107 from Palestine), located in 23 farms in Israel and 15 farms in Palestine. Farms were selected to represent the geographic distribution of the horse population in Palestine and Israel. One hundred and eighty-four horses from 13 farms in Israel and 26 horses from 1 farm in Palestine were sampled twice, once during July to August 2014 and again during November to December 2014. Data for each horse were collected from farm managers and included sex, breed, age, housing, and recent health condition. None of the horses had a history of recent febrile disease, and all had rectal temperature within normal limits (36.5–38.5°C). None of the tested horses was imported in the last year, and importation of horses to Israel and Palestine is very limited. None of the tested horses was vaccinated against WNV.

Samples were also collected from 124 donkeys (49 in Israel and 75 in Palestine), located in three farms in Israel and in nine farms in Palestine. Blood samples were also collected from 50 goats, 45 sheep, and 35 camels in three regions of Palestine (Nablus, Jericho, and Jenin). Blood was collected from the jugular vein of each animal into a sterile vacuum tube without an anticoagulant agent. Sera were obtained from clotted blood samples by centrifugation (3000 *g* for 8 min) and stored at −20°C until use.

Blood collections were performed under owners' consent, and the study was approved by the Internal Ethics Review Committee of the Koret School of Veterinary Medicine, The Hebrew University.

Competitive ELISA

IgG levels in serum samples were determined using a commercial ELISA kit (ID Screen® West Nile Competition Multi-species; IDvet Innovative Diagnostics), according to the manufacturer's instructions.

Serum neutralization

Serum neutralization (SN) test was performed on 200 samples from horses from Israel and only on 20 samples from animals (7 horses, 7 camels, 2 each of donkeys, goats, and sheep) in Palestine. (Unfortunately, due to technical problems, not enough serum was left from animals from Palestine, and therefore, we did not succeed to retest more samples). Double dilutions from 1:4 to 1:512 in duplicates of 50 μL of each serum sample were made in 96-well plates in Eagle's medium (according to standard of WRL, Pirbright). Negative and positive (weak 1:16, strong 1:512) anti WNV serum was used for control. Fifty microliters (10^2 median tissue culture infective dose [$TCID_{50/50}$] μL) of the challenge virus—WN98 (GenBank acc. no. AY033388) (Banet-Noach et al. 2003) were added to each well and allowed to stand for 30 min at 37°C. One hundred microliters of the Serum-Ag mixture was then added to 96 cell culture plates containing 100 μL of Vero E6 cells, and the plates were placed in a 37°C incubator (5% CO_2) for 4 days, when they were inspected microscopically for cytopathic effect (CPE). The neutralizing antibody titer of the serum was calculated as the highest dilution at which complete neutralization of CPE was observed.

Collection of environmental data

Time series of data from the Moderate Resolution Imaging Spectroradiometer (MODIS) on board NASA's Terra (launched December 1999) and Aqua (launched May

2002) polar-orbiting satellites offer the potential to capture environmental thermal and vegetation seasonality over land. MODIS has 36 spectral bands, with spatial resolutions of 250 meters (bands 1–2), 500 meters (bands 3–7), and 1 km (bands 8–36). The relevant terrestrial MODIS datasets are already being archived and are ready to use by a geographic information system (GIS) based tool that has already been constructed. We retrieved the following environmental variables from MODIS for the years 2001–2010: NDVI—Normalized Difference Vegetation Index, 250-meter spatial resolution; LSTD—Day land surface temperatures at 1-km spatial resolution; and LSTN—Night land surface temperatures at 1-km spatial resolution.

For each variable we recorded the average value. A GIS layer was constructed for each variable.

In addition, we used GIS layers of altitude, multiannual yearly average precipitation, and yearly average salinity. The multiannual data were recorded by the Israeli Meteorological Service. All environmental variables were joined with the WNV data based on spatial location.

Statistical analysis

Seroprevalence was estimated separately for each of the study populations (Israel, Palestine). Statistical significance of the differences in the prevalence between these populations was assessed by the two-sided chi-squared test. Risk factors associated with exposure to WNV were assessed for each population separately. Association with nominal independent variables was assessed using the chi-squared test, and odds ratios (ORs) were calculated. Association with quantitative parameters was assessed using *t*-test. Association between variables was considered statistically significant when p value was <0.05. All significant parameters in the univariable analysis were included in a multivariable analysis, using a forward stepwise model. The data for the Israeli and Palestinian populations were also analyzed using generalized estimating equation with a logit link function, with the farm set as a subject (*i.e.*, random variable) and with an exchangeable working correlation matrix. The analysis was performed using SPSS 22.0® and WinPepi 11.43® statistical software.

Results

Study population

A total of 460 horses were sampled, the population distributed almost equally between male and female horses, with 225 mares (48.9%), 234 males (50.9%), and for 1 the sex was unknown. The age of the horse was indicated for 413 horses. Ages ranged between 6 months and 30 years, with a mean age of 10.1 ± 5.5 years. Thirty-three horses were younger than 3 years old (8%), 315 (76.3%) horses were between 4 and 15 years old, and 65 (15.7%) horses were older than 16 years. Half of the horses (51.1%) were local horses ($n = 235$), 84 (18.3%) were Quarter horses, 22 (4.8%) were Arabians, and few horses were from other breeds; for 47 (10.2%) horses data were not available. One hundred and twenty-nine horses were housed in stalls (28%), 91 (19.8%) were kept in paddocks and 132 (28.7%) were kept in pasture; for 108 (23.5%) data were not available.

A total of 124 donkeys were sampled, 53 (42.7%) females and 71 (57.3%) males. The age was known for 64 donkeys. Ages ranged between 6 months and 25 years, with a mean age of 7.8 ± 5.2 years. Thirteen donkeys were under 3 years old (20.3%), 47 were between 4 and 15 years old (73.4%), and 4 were over 16 years old (6.3%).

Thirty-five camels were sampled in three districts of Palestine, 28 females (80%) and 7 males (20%). Forty-five

Table 1. Univariable Analysis of Risk Factors Considered for an Association with West Nile Virus Seropositivity in Equids Sampled in Israel and Palestine

Variable	*Category* (n)	*No. of carriers (%)*	*OR (95% CI)*	p *Value (Fisher's two tailed)*	p *Value* (χ^2)
	Israel	340/402 (84.6)	5.79 (3.9–8.59)	**<0.001**	**<0.001**
	Palestine	90/185 (48.6)	Ref.		
Area	Northern Israel	117/152 (77)	4.86 (2.52–9.37)	**<0.001**	**<0.001**
	Central Israel	103/118 (87.3)	9.99 (4.66–21.39)	**<0.001**	
	Southern Israel	54/63 (85.7)	8.73 (3.61–21.1)	**<0.001**	
	Golan Heights	46/48 (95.8)	33.45 (7.46–150.02)	**<0.001**	
	Northern Palestine	11/33 (33.3)	0.73 (0.3–1.78)	0.649	
	Jerusalem Mountains	33/54 (61.1)	2.29 (1.07–4.9)	0.054	
	Great Rift Valley	44/65 (67.7)	3.05 (1.45–6.42)	**<0.001**	
	Southern Palestine	22/54 (40.7)	Ref.		
Species	Horse	380/460 (82.6)	7.03 (4.57–10.82)	**<0.001**	**<0.001**
	Donkey	50/124 (40.3)	Ref.		
	Mule	0/3 (0)	0.21 (0.02–2.81)	0.278	
Sex	Female	200/279 (71.7)	0.86 (0.6–1.24)	0.456	0.456
	Male	229/307 (73.2)	Ref.		

Significant numbers appear in *bold*.
CI, confidence interval; OR, odds ratio.

sheep and 50 goats were also sampled in three districts of Palestine, 83 females (87.4%) and 12 males (12.6%).

Seroprevalence of WNV in equids in Israel and Palestine

Four hundred and thirty of 587 horses, donkeys, and mules sampled in Israel and Palestine were seropositive for WNV by competitive ELISA (cELISA; 73%, 95% confidence interval [CI]: 69.5–76.8).

One hundred and ninety-two of the 200 horses (96%) from Israel that were WNV seropositive by cELISA were also positive by the SN method. Seven of seven seropositive horses and seven of seven seropositive camels from Palestine were also positive by the SN method. However, only one of two seropositive goats, one of two seropositive donkeys, and none of two seropositive sheep were found positive.

Risk factors for exposure to WNV of equids in Israel and Palestine

Seroprevalence in Israel (340/402 animals, 84.6%) was significantly higher ($p<0.001$) than in Palestine (90/185 animals, 48.6%). Seroprevalence in horses (380/460 horses, 82.6%) was significantly higher ($p<0.001$) than in donkeys and mules (50/127 animals, 39.3%) (Table 1). Dividing Israel and Palestine into eight geographical areas demonstrated lower seroprevalence in all Palestinian provinces, except in the Great Rift Valley area (Jerico and the Dead Sea), that had higher seroprevalence, similar to Israel (Table 1 and Fig. 1). The mean age of WNV seropositive horses was significantly higher compared with seronegative horses (Table 2). Environmental factors that were found significantly associated with WNV exposure were: population in the sampled settlement, the mean LSTD and mean LSTN, the average altitude, and the average salinity (Table 2). Multivariable statistical analysis showed the geographic area, animal species, age, mean LSTD, and average altitude to correlate with WNV seroprevalence (Table 3).

Risk factors for exposure to WNV of horses and donkeys in Israel and Palestine

Inspection of only the horse population revealed that WNV seroprevalence in Israel was higher than in Palestine and that the areas of central Israel ($p<0.001$), the Golan Heights ($p=0.01$), and southern Israel ($p=0.017$) demonstrated significantly higher seroprevalence than other areas in Israel and Palestine (Table 4). Univariable analysis for potential risk factors showed association between horse age, breed, housing and coat color, and exposure to WNV (Tables 2 and 4). Young horses (under 3 years) were less likely to carry antibodies against WNV ($p<0.001$), and the mean age of seropositive horses was significantly higher than seronegative horses (10.7 and 6.6 years, respectively, $p<0.001$, $N=413$). Seroprevalence varied in different horse breeds. Quarter horses were four times more likely to be exposed to WNV than mixed breeds ($p<0.001$), while Tinkers ($p=0.034$) and Arabians ($p<0.001$) had lower seroprevalence than mixed breeds. Horses housed in stalls or paddocked showed higher WNV exposure than horses in pastures ($p<0.001$). Colored horses (Pintos and Appaloosas) had lower seroprevalence than solid-colored horses

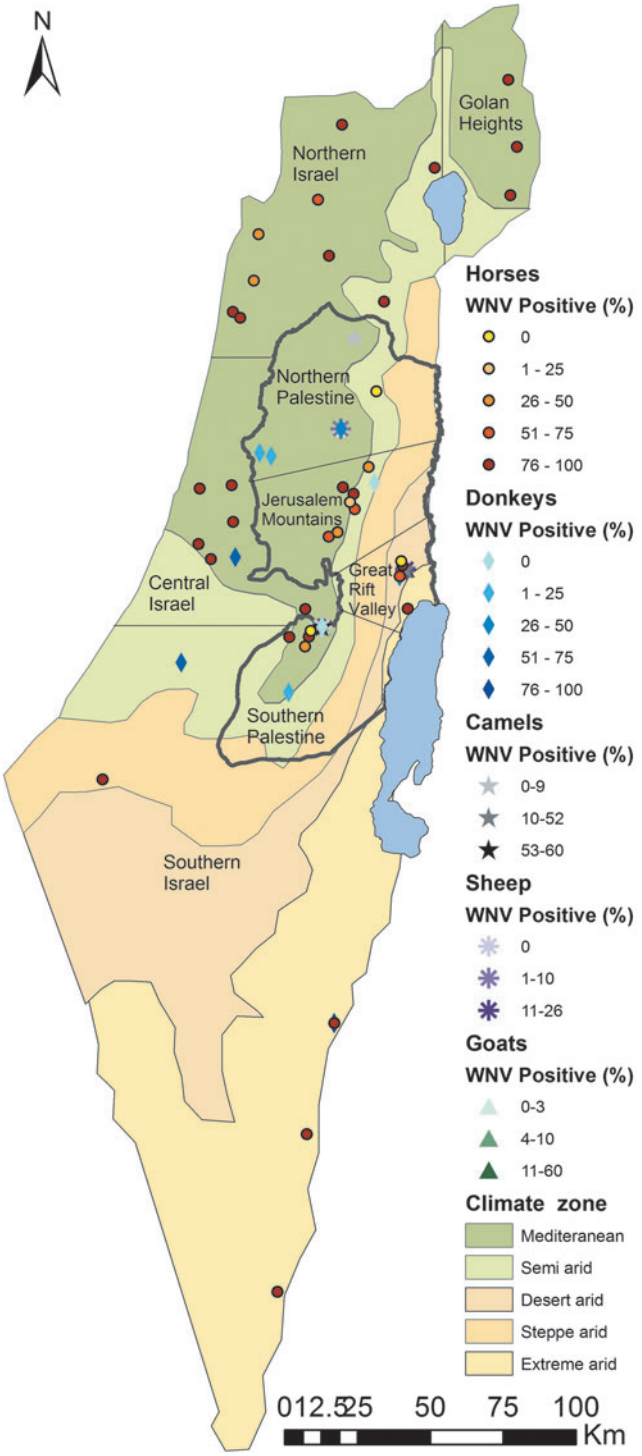

FIG. 1. WNV seroprevalence in various animal species in Palestine and Israel. WNV seroprevalence levels and geographic distribution of horses and donkeys farms and of camels, sheep and goats sampled during 2014–2015.

($p<0.001$) (Table 4). Environmental factors that were found significantly associated with WNV exposure were as follows: mean NDVI, mean LSTD, average altitude, and average salinity (Table 2). Multivariable analysis included only horses from Israel, since information regarding housing and coat color was not available for the horses from Palestine. Factors

Table 2. Univariable Analysis of Risk Factors Considered for an Association with West Nile Virus Seropositivity in Equines Sampled in Israel and Palestine and in Horses and Donkeys Separately

	Mean positive (n)	*Mean negative* (n)	p (*t-test*)	*Mean difference (95% CI)*	*Standard error difference*
Equids					
Age	10.58 (373)	6.92 (104)	**<0.001**	3.66 (2.51 to 4.8)	0.584
Population	13,374 (430)	31,847 (157)	**<0.001**	−18,473 (26,120 to 10,826)	3879
Average rain	508 (430)	529 (157)	0.272	−20.75 (−54.23 to 12.73)	17.02
Mean NDVI	0.035 (430)	0.034 (157)	0.681	0.0005 (0.0019 to 0.0029)	0.0012
Mean LSTD	30.37 (430)	29.31 (157)	**<0.001**	1.05 (0.55 to 1.55)	0.254
Mean LSTN	15.39 (430)	14.86 (157)	**0.014**	0.53 (0.11 to 0.95)	0.216
Average altitude	234.61 (430)	377.08 (157)	**<0.001**	−142.74 (−211.53 to 73.41)	33.26
Average salinity	1.72 (430)	1.44 (157)	**0.014**	0.27 (0.05 to 0.5)	0.112
Horses					
Age	10.72 (350)	6.62 (63)	**<0.001**	4.09 (2.67 to 5.51)	0.72
Population	10,162 (380)	7892 (80)	0.521	2299 (−4741 to 9340)	3582
Average rain	517 (380)	566 (80)	0.062	−48.58 (−99.65 to 2.48)	25.98
Mean NDVI	0.035 (380)	0.041 (80)	**0.005**	−0.0055 (−0.0093 to 0.0017)	0.0019
Mean LSTD	30.21 (380)	28.52 (80)	**<0.001**	1.68 (1.01 to 2.36)	0.343
Mean LSTN	15.35 (380)	14.90 (80)	0.13	0.44 (−0.13 to 1.03)	0.296
Average altitude	249.53 (380)	365.08 (80)	**0.008**	−115.55 (−200.93 to 30.16)	43.44
Average salinity	1.70 (380)	1.37 (80)	**0.021**	0.33 (0.05 to 0.60)	0.194
Donkeys					
Age	8.5 (23)	7.3 (41)	0.406	1.11 (−1.56 to 3.79)	1.35
Population	37,786 (50)	56,528 (74)	**0.015**	−18,742 (−33,721 to 3763)	7566
Average rain	437.5 (50)	493.91 (74)	**0.048**	−56.41 (−112.44 to 0.38)	28.3
Mean NDVI	0.311 (50)	0.278 (74)	**0.002**	0.0032 (0.0012 to 0.0053)	0.001
Mean LSTD	31.54 (50)	30.12 (74)	**<0.001**	1.41 (0.65 to 2.17)	0.384
Mean LSTN	15.75 (50)	14.81 (74)	**0.01**	0.93 (0.22 to 1.65)	0.361
Average altitude	121.19 (50)	386.13 (74)	**<0.001**	−264.94 (−383.72 to 146.16)	59.99
Average salinity	1.88 (50)	1.5 (74)	0.054	0.38 (−0.006 to 0.771)	0.196
Animals in Palestine					
Population	33,846 (65)	65,268 (134)	**<0.001**	−31,422 (−40,552 to 22,292)	4629
Average rain	233.46 (65)	455.59 (134)	**<0.001**	−222.13 (−271.99 to 172.27)	25.26
Mean NDVI	0.027 (65)	0.026 (134)	**0.016**	0.00048 (0.00009 to 0.00086)	0.0002
Mean LSTD	33.65 (65)	30.26 (134)	**<0.001**	3.39 (2.61 to 4.16)	3.39
Mean LSTN	18.31 (65)	15.58 (134)	**<0.001**	2.73 (2.11 to 3.35)	0.31
Average altitude	−112.02 (65)	368.76 (134)	**<0.001**	−480.79 (−593.23 to 368.34)	56.94
Average salinity	3.25 (65)	1.83 (134)	**<0.001**	1.42 (1.1 to 1.73)	0.16

Environmental factors include the population in the settlement sampled, average annual rainfall, mean vegetation index (NDVI), mean LSTD and LSTN, geographical height, and average land salinity.
Significant numbers appear in *bold*.
LSTD, land surface temperature during the day; LSTN, land surface temperature during the night.

that were found to significantly associate with WNV seroprevalence were the breed, age, mean LSTD, and average altitude (Table 3).

WNV was also more prevalent in donkeys in Israel (30/49 donkeys, 61.2%) than in Palestine (20/75 donkeys, 26.7%). Donkeys in Israel were more likely to be exposed to WNV than in Palestine (OR: 4.34, 95% CI: 2.02–9.31, $p < 0.001$). Donkeys in Southern Palestine and the Jerusalem mountains had lower seroprevalence than in other areas in Israel and Palestine ($p < 0.001$) (Table 5). Environmental factors that were found significantly associated with WNV exposure were as follows: population in the sampled settlement, average rain, mean NDVI, mean LSTD, mean LSTN, and average altitude (Table 2). Multivariable analysis revealed Israel population and average altitude to be significantly associated with higher WNV seroprevalence (Table 3).

Seroprevalence and risk factors for WNV exposure in other animals in Palestine

Fourteen of 95 (14.7%) sheep and goats sampled in Palestine were seropositive for WNV. Seroprevalence did not differ statistically between sheep and goats or between male and female animals. Animals were sampled in three locations in Palestine (Bethlehem, Jericho, and Nablus). Seroprevalence in Jericho was higher than in other locations (OR: 11, 95% CI: 3.08–39.26, $p < 0.001$).

WNV seroprevalence in camels was 40% (14/35 camels). Camels were sampled in three locations in Palestine (Bethlehem, Jericho, and Jenin). Seroprevalence in Jenin was lower than in other locations (OR: 0.08, 95% CI: 0.01–0.7, $p = 0.04$).

Univariable statistical analysis of the different animal species sampled in Palestine revealed significant association among animal species ($p < 0.001$), sampling location

Table 3. List of Factors That Were Found to Be Significantly Associated with West Nile Virus Seropositivity in Equines Sampled in Israel and Palestine in the Multivariable, Forward Stepwise Analysis, As Well As in Horses and Donkeys Separately

	p	*OR*
Equids		
Area (Jerusalem mountains)	0.002	0.063
Species (horse)	<0.001	24.128
Age	<0.001	1.169
Mean LSTD	<0.001	1.81
Average altitude	0.07	1.002
Horses		
Breed (mixed)	0.018	21.153
Breed (Quarter horse)	0.033	14.079
Age	<0.001	1.263
Mean LSTD	<0.001	2.868
Average altitude	0.018	1.004
Donkeys		
Israel	<0.001	4.342
Population	0.025	1
Average altitude	0.001	0.996

All factors that were found significant in the univariable analysis were included.

($p<0.001$), animal sex ($p=0.003$), and WNV seroprevalence (Table 6). Seroprevalence in horses was higher than in other animals (OR: 6.86, 95% CI: 3.26–14.44, $p<0.001$), and seroprevalence in equines was higher than in small ruminants and camels (OR: 4.33, 95% CI: 2.31–8.09, $p<0.001$). Seroprevalence in Jericho was 56.7%, significantly higher than in other locations (Table 6). Seroprevalence in male animals was higher than in female animals (Table 6). Animal species and sex were also found to be significantly associated with WNV exposure in the multivariable analysis ($p<0.001$ and $p=0.027$, respectively). All the tested environmental factors were found to be significantly associated with WNV exposure (Table 2). These factors were excluded from the multivariable analysis since they were not distributed equally and there were intervariable associations.

Annual incidence of WNV in horses in Israel and Palestine

To evaluate the annual/seasonal incidence of WNV, 210 individual horses were sampled both at the beginning and at the end of the mosquito season (during July to August and during November to December 2014). Of these horses, 174 were seropositive for WNV at the first sampling (82.8%), and all remained seropositive. Three of the seronegative horses converted to positive when resampled (8.5%). All recent infections during the mosquito season were reported in horses in Palestine. In Palestine 26 horses were sampled twice, all in the same farm. Seventeen horses were seropositive in both time points. Seroconversion occurred in three of nine (33%) originally negative horses.

Discussion

The findings in this study demonstrate for the first time that animals in Palestine were exposed to WNV. The cELISA that was used detects antibodies directed against the envelope protein of the WNV. This test gives positive results around 10 days after exposure, and the serum of affected horses can remain positive for years (Durand et al. 2002). One limitation of the ELISA method to assess WNV seroprevalence is its lack of specificity due to cross-reactivity between antibodies directed against WNV and other *Flaviviruses* such as St. Louis encephalitis virus (SLEV), Japanese encephalitis virus (JEV), Tick-borne encephalitis virus (TBEV), Usutu virus (USUV), and Zika virus. To the best of our knowledge, except for USUV which was identified in very few mosquito pools in Israel (Lustig Yaniv, personal communication), these pathogens are not endemic in Israel and in Palestine. Although unlikely, we also cannot exclude the possibility that animals that were imported from endemic areas are seropositive as was recently demonstrated in six Israeli travelers that were diagnosed with Zika virus after travelling into Zika virus endemic areas (Lustig et al. 2016). To test for possible cross-reactivity, 220 of the ELISA seropositive samples were retested using SN. The finding that 96% of 200 WNV ELISA seropositive samples from horses in Israel, 7/7 samples from horses in Palestine, and 7/7 samples from camels in Palestine were also seropositive by the SN method further support the assumption that they were exposed to WNV. In contrary, only one of two goats, one of two donkeys, and none of two sheep that were seropositive in WNV ELISA were also seropositive by the SN method. It is therefore possible that small ruminants in Palestine were seropositive to other *Flaviviruses*; however, the sample size is too small to draw conclusive conclusions and further works should be done in the future to test this. Unfortunately, due to technical problems, no serum was left from those animals to retest more of them by the SN method.

Our findings indicate that WNV circulated in Palestine in the past. Seroconversion that occurred in three of nine serologically negative horses, in Palestine, in the summer of 2014, is an indication for the activity of WNV during that time. None of the tested seronegative horses in Israel became seropositive in the end of the summer of 2014. According to the Israeli Ministry of Health (www.health.gov.il/Subjects/disease/WNF/Pages/StatusReport2014.aspx in Hebrew), during the same months in 2014, 20 people had WNF (11 of which were confirmed), which was much less than during the same months in 2013. Therefore, it is possible that fewer horses were infected in 2014 and since most were already seropositive, clinical affection did not occur. Although much information on WNF is available from Israel and a broad range of control measures to prevent human infection has been implemented (Anis et al. 2014), this is not the situation in Palestine. It is therefore probable that WNF in humans in Palestine is either underdiagnosed or underreported and attention should be drawn to this important disease, especially in high-risk elderly population.

Prevalence of WNV antibodies in equids (horses and donkeys) in Palestine was lower than in Israel, except for equids in the Great Rift Valley area. This can be a true lower activity of the virus in Palestine or the result of different management or demographic parameters that were not available for us in this study. In Jordan, which has a border with both Palestine and Israel, seroprevalence among horses was 24.9%; higher prevalence was found in the Jordan Valley and Balqa region (Abutarbush and Al-Majali 2014). This is different than the much higher prevalence that was found in horses in Israel, both in this study and previously

Table 4. Univariable Analysis of Risk Factors Considered for an Association with West Nile Virus Seropositivity in Horses Sampled in Israel and Palestine

Variable	*Category* (n)	*No. of carriers (%)*	*OR (95% CI)*	p *Value (Fisher's two tailed)*	p *Value* (χ^2)
	Israel	310/353 (87.8)	3.81 (2.29–6.34)	**<0.001**	**<0.001**
	Palestine	70/107 (65.4)	Ref.		
Area	Northern Israel	117/152 (77)	1.11 (0.44–2.8)	0.811	**<0.001**
	Central Israel	91/95 (95.8)	7.58 (2.06–27.91)	**<0.001**	
	Southern Israel	36/37 (97.3)	12 (1.42–101.22)	**0.017**	
	Golan Heights	46/48 (95.8)	7.67 (1.5–39.26)	**0.01**	
	Northern Palestine	0/1 (0)	—	0.276	
	Jerusalem Mountains	33/53 (62.3)	0.55 (0.2–1.5)	0.324	
	Great Rift Valley	36/46 (78.3)	1.2 (0.4–3.57)	0.781	
	Southern Palestine	21/28 (75)	Ref.		
Sex	Female	184/225 (81.8)	0.9 (0.55–1.45)	0.713	0.713
	Male	195/234 (83.3)	Ref.		
Age	Under 3 years	15/33 (45.5)	Ref.		**<0.001**
	Four to 15 years	274/315 (87)	8.02 (3.79–16.98)	**<0.001**	
	Over 16 years	61/65 (93.8)	18.3 (5.46–61.34)	**<0.001**	
Breed	Mixed	193/235 (82.1)	Ref.		**<0.001**
	Thoroughbred	5/5 (100)	2.42 (0.17–34.66)	0.59	
	Missouri Foxtrot	4/4 (100)	1.98 (0.14–27.54)	1	
	Paint Horse	12/14 (85.7)	1.31 (0.3–5.74)	1	
	Quarter Horse	80/84 (95.2)	4.35 (1.52–12.47)	**<0.001**	
	Shire	1/1 (100)	—	1	
	Warmblood	11/11 (100)	5.05 (0.33–77.54)	0.219	
	Pony	12/14 (85.7)	1.31 (0.3–5.74)	1	
	Tinker	0/2 (0)	—	**0.034**	
	Tennessee Walking Horse	14/14 (100)	6.37 (0.41–99.01)	0.136	
	Appaloosa	5/7 (71.4)	0.54 (0.11–2.57)	0.614	
	Arabian	10/22 (45.5)	0.18 (0.07–0.44)	**<0.001**	
Housing	Stall	118/129 (91.5)	2.76 (1.31–5.82)	**<0.001**	**0.001**
	Paddock	86/91 (94.5)	4.42 (1.64–11.91)	**<0.001**	
	Pasture	105/132 (79.5)	Ref.		
Color	Dark	175/192 (91.1)	4.25 (2.06–8.79)	**<0.001**	**<0.001**
	Light	94/106 (88.7)	3.24 (1.45–72)	**<0.001**	
	Colored	46/65 (70.8)	Ref.		

Significant numbers appear in *bold.*

Table 5. Univariable Analysis of Risk Factors Considered for an Association with West Nile Virus Seropositivity in Donkeys Sampled in Israel and Palestine

Variable	*Category* (n)	*No. of carriers (%)*	*OR (95% CI)*	p *Value (Fisher's two tailed)*	p *Value* (χ^2)
	Israel	30/49 (61.2)	4.34 (2.02–9.31)	**<0.001**	**<0.001**
	Palestine	20/75 (26.7)	Ref.		
Area	Central Israel	12/23 (52.2)	25.09 (3.02–208.41)	**<0.001**	**<0.001**
	Southern Israel	18/26 (69.2)	51.75 (6.19–432.56)	**<0.001**	
	Northern Palestine	11/32 (34.4)	12.05 (1.49–97.15)	**<0.001**	
	Jerusalem Mountains	0/1 (0)	—	1	
	Great Rift Valley	8/18 (44.4)	18.4 (2.13–159.26)	**<0.001**	
	Southern Palestine	1/24 (4.2)	Ref.		
Sex	Female	16/53 (30.2)	0.47 (0.22–0.99)	0.064	0.064
	Male	34/71 (47.9)	Ref.		
Age	Under 3 years	4/13 (30.8)	Ref.		0.825
	Four to 15 years	18/47 (38.3)	1.4 (0.39–4.98)	0.751	
	Over 16 years	1/4 (25)	0.75 (0.08–7.21)	1	

Significant numbers appear in *bold.*

Table 6. Univariable Analysis of Risk Factors Considered for an Association with West Nile Virus Seropositivity in Various Animal Species Sampled in Palestine

Variable	*Category* (n)	*No. of carriers (%)*	*OR (95% CI)*	p *Value (Fisher's two tailed)*	p *Value* (χ^2)
District	Bethlehem	5/37 (13.5)	1.95 (0.5–7.7)	0.477	**<0.001**
	Jenin	1/11 (9.1)	1.25 (0.14–11.33)	1	
	Jericho	55/97 (56.5)	16.37 (5.53–48.45)	**<0.001**	
	Nablus	4/54 (7.4)	Ref.		
Species	Horse	28/41 (68.3)	14 (4.8–40.81)	**<0.001**	**<0.001**
	Donkey	10/27 (37)	3.82 (1.22–12.01)	**0.038**	
	Mule	0/2 (0)	n/a	1	
	Camel	14/35 (40)	4.33 (1.47–12.76)	**0.009**	
	Goat	8/50 (16)	1.24 (0.4–3.84)	0.778	
	Sheep	6/45 (13.3)	Ref.		
Sex	Female	42/153 (27.5)	Ref.	**0.004**	**0.003**
	Male	24/47 (51.1)	2.76 (1.41–5.38)		

Significant numbers appear in *bold*.

(Aharonson-Raz et al. 2014), which may be the result of environmental factors and the abundance of mosquitoes which are required for the transmission cycle.

Seroprevalence in horses, in this study, was significantly higher than in donkeys and mules. In recent studies from Tunisia (Bargaoui et al. 2015) and Northwest Senegal (Davoust et al. 2016), no statistically significant difference was observed between horses and donkeys. The reason for this difference in our study is not known, but since the seroprevalence among horses in Israel is very high, making it difficult to find seronegative horses, donkeys may be used as sentinels for the virus activity.

In Palestine, WNV antibodies were detected not only in equids but also in small ruminants. Unfortunately, WNV infections were not confirmed in small ruminants; therefore, it is possible that infection was caused by another *Flavivirus,* which could explain the differences in seroprevalence rates between studies. The seroprevalence in small ruminants (14.7%) and in camels (40.0%) is much higher than what was recently found in Northwest Senegal (0% in sheep, 6.9% in goats, and 0% in cattle), although high seroprevalence was found in that study in horses, in donkeys, and in dogs demonstrating viral activity (Davoust et al. 2016). It is also higher than the seroprevalence that was found in cattle and sheep in Turkey (4% in cattle, 1% in sheep); low seroprevalence was also found in ass mules (2.5%) in this study, although as in Senegal higher seroprevalence was found in other mammals (37.7% in dogs, 13.5% in horses, and 20.4% in humans) (Ozkul et al. 2006). In addition, in a serosurvey from Trinidad, antibodies to WNV were only found in horses (17.2%) and were not found in cattle, sheep, goats, and other animals (Thompson et al. 2012). Similarly, in Brazil, antibodies against WNV and against other *Flaviviruses* were not found in sheep, therefore, the authors concluded that sheep fail to develop antibodies for Brazilian *Flaviviruses*, or that they may not attract local mosquito vectors (Pauvolid-Correa et al. 2014).

The seroprevalence that was found in our study further indicate activity of the virus in Palestine and even though horses or alternative animal species (dogs, cattle, camels, etc.) do not generally develop high viremia after WNV infection, WNV infection induces antibody responses. The use of animals other than horses as sentinels was previously suggested following a serosurvey in Morocco where high seroprevalence was found in military working dogs (Durand et al. 2016). Determining seroprevalence and more importantly seroconversion in animals other than horses may be required in areas where the seroprevalence in horses is very high or where many horses are vaccinated against WNV preventing their use as sentinels for the virus activity.

Multiple factors impact the transmission and distribution of WNV. Among other drivers, weather conditions have direct and indirect influences on vector competence, on the vector population dynamic and on the virus replication rate within the mosquito (Paz 2015). Ambient temperature plays an important role in viral replication rates and transmission of WNV (Paz 2015). Above-average precipitation might lead to a higher abundance of mosquitoes and increase the potential for disease outbreaks in humans (Paz 2015). In a previous study, lower spring precipitation level was revealed during the years with increased WNV human cases and 1 year before them compared to the rest of the years, supporting the notion of association between WNV epidemics and a prior dry spring season (Aharonson-Raz et al. 2014). In this study, the two environmental factors that were found to be significantly associated with WNV exposure in the multivariable analysis for equids and specifically for horses were the mean LSTD and average altitude. Higher WNV seroprevalence was found in warmer and lower areas, which correlate with the vector preference (Paz 2015). This has an important significance in exposure prevention efforts during the years and specifically between years.

Conclusions

Antibodies against WNV were found, for the first time, in several domestic animals in Palestine indicating viral activity in the past. Furthermore, seroconversion was demonstrated in three of nine seronegative horses indicating that WNV was circulating in Palestine in the summer of 2014. Control measures to prevent human infection should be implemented since it is probable that WNF in human is underdiagnosed

or underreported in Palestine. Anti WNV antibodies were detected in small ruminants, camels, and donkeys indicating that those species can be used as sentinels for WNV activity in areas where most horses are either seropositive or vaccinated.

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