The possible role of mites and ticks as vectors of West Nile Virus
Argasid Ticks as Possible Vectors of West Nile Virus in Israel

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ABSTRACT

Mites and soft ticks collected directly from wild and domestic birds and their nests were tested for the presence of West Nile virus (WNV). The cattle egret argas, Argas arboreus, was collected from the nests of seven cattle egret colonies. Out of 1,000 A. arboreus pools examined, 16 were positive for WNV based on RT-PCR technique. The positive pools were from four nesting colonies of birds. Out of 37 cattle egret squabs examined, 37.8% had serum-neutralizing antibodies to WNV. WNV RNA was also detected in one out of 15 pools of R. turanicus, in one out of 21 pools of O. sylviarum, and in one out of 18 pools of D. gallinae, while 63 pools of A. reflexus, 11 of R. sanguineus, and 30 of Hyalomma spec. were negative. The role of mites and ticks in maintaining the endemic state of WNV in Israel is discussed. Key Words: West Nile Virus—RT-PCR—Ornithonyssus sylviarum—Dermanyssus gallinae—Argas arboreus—Rhipicephalus turanicus—Israel.

INTRODUCTION

West Nile fever (WNF) is endemic in Israel. The virus was first isolated in 1953 by Bernkopf et al. during an outbreak of the disease. Additional outbreaks occurred in 1980 and 2000 (Katz et al. 1989; Chovers et al. 2001; Weinberger et al. 2001). From August to October 2000, a major epidemic of WNF occurred in the human population. Cases were distributed throughout the country, while the highest incidence was observed in central Israel, which is the most populated area. The disease was confirmed in 417 patients of whom 326 were hospitalized. Twenty-seven people, mainly in the 70–90-year age group, died from WNF complications (Chovers et al. 2001; Weinberger et al. 2001). The main mosquito vectors in Israel are Culex perexiguus and Culex pipiens (Samina et al. 1986). Several animal reservoirs have been identified in Israel, and the virus has been isolated from pigeons, storks, gulls, crows, turtle doves and wagtails (Nir et al. 1967, 1972; Malkinson and Banet 2002; Banet-Noach et al. 2003b). During 1997–1998, WNV was reported for the first time as causing illness and high mortality among young domestic geese in Israel (Malkinson et al. 1998). WNV was also isolated from migrating storks and horses (Malkinson et al. 2002; Steinman et al. 2002). Most cases of WNF occur in Israel between
August and October, corresponding to the late summer peak of culicine mosquito populations. It is assumed that migratory birds introduce the virus annually to the country and local mosquitoes transmit it to native wild and domestic birds, thus perpetuating viral transmission. Moreover, transovarial transmission in mosquitoes or maintenance of the virus in local bird populations by ticks could also be a major factor contributing to the endemic state of the disease.

The aim of this study was to examine whether blood-sucking mites and ticks, which parasitize humans and birds, harbor WNV and might be potential vectors for the disease in Israel and whether they play a role in maintaining WNV through the winter months.

MATERIALS AND METHODS

Sampling of mites and ticks

Samples were collected from 38 localities in Israel where, according to the Ministry of the Environment, WNV activity was observed in people and/or mosquitoes in the years 2000–2003. (www.environment.gov.il/Environment/bin/en.jsp?enPage=HomePage).

Twenty-five pigeons and 55 laying hens from six poultry houses were examined for ectoparasites on their feathers and skin.

Two hundred ten cattle egret nests from six colonies (each with 25–40 nests) were studied. In addition, 36 pigeon and 19 sparrow nests, as well as seven soil samples from two commercial goose farms and 43 samples from 14 poultry houses were examined. Mites, ticks and other ectoparasites and nest inhabitants were isolated by the Berlese funnel method, in which the arthropods escape from the heat and dryness produced by an electric bulb and are collected at the bottom of a funnel (Messenger et al. 2000). Nesting material from cattle egrets was also examined by shaking the branches and examining the loose material under a magnifying glass.

Ten localities around human habitations and dog kennels and open areas with typical tick biotopes were examined using a white flannel flag measuring 2 × 1.5 m. Ten strips of vegetation, measuring 10 m long, were examined. Ticks which are attracted to hairy, moving objects attach themselves to the flannel and were collected when trying to escape (Ginsberg and Ewing 1989).

Mites and ticks were collected throughout the year.

Virus isolation and neutralization

Pools of 10 ticks or approximately 100 mites were stored at −70°C until examination. The arthropods were placed into 10% fetal calf serum (Biological Industries Ltd., Israel) + 10% of antibiotics mixture: 10,000 units of penicillin G sodium salt, 10 mg of streptomycin sulfate, 25 µg of amphotericin B per mL ("Pen-Strep-Ampho" Biological Industries Ltd.) PBS solution, and homogenized by hand for at least 1 min in an Eppendorf tube (1.5 mL) using plastic microtube pestles. Samples were stored at −70°C until examination. Before use, the samples were centrifuged at 3,500 rpm for 5 min, and the supernatant was then collected for RNA extraction and virological examination.

Cattle egrets were bled using heparinized syringes. The plasma was separated by centrifugation at 1,500 rpm for 10 min and kept frozen at −70°C. Birds were then euthanized by CO2 inhalation, and necropsies were performed aseptically. Brains were removed and homogenized by grinding them in PBS, centrifuged at 3,500 rpm for 5 min and the supernatants filtered (0.22 nm). Samples were kept frozen at −70°C.

Virus neutralization, viremia, and virus isolations were performed as previously described (Banet-Noach et al. 2003a).

RNA extraction, RT-PCR, and real-time RT-PCR

RNA from the ticks and mites was extracted with RNEasy Kit (Qiagen Gmbh, Hilden, Germany) according to manufacturer’s instructions and resuspended in 30 µL of RNase-free water and stored at −70°C. Direct reverse transcriptase–polymerase chain reaction (RT-PCR) was performed as described previously (Banet-Noach et al. 2003a).

Real-Time RT-PCR was performed with the TaqMan® One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystem Inc., Foster City, CA, USA) following the manufacturer’s instructions, with the exception that the cycling parameters were 50°C (2 min), 95°C (10 min), and 40 cycles of 95°C (15 s), 60°C (1 min). Each sample was amplified in a volume of 25 µL, containing 12.5 µL of TaqMan Master Mix, 200 nM of each primer, 50 nM of each probe, and 10 µL of template. The amplification was performed in a 96-well optical plate format using the Applied Biosystem 7500 Fast Real-Time PCR System (Applied Biosystem Inc., Foster City, CA, USA).
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City, CA) using the probe and primers designed for the WNV envelope gene by Lanciotti et al. (2002). This group established a positive cutoff of a dilution of $10^{-6}$ of the seed virus (equivalent of 1 PFU/mL).

RESULTS

Argas arboreus was collected from all six colonies of cattle egrets (Bubulcus ibis), located at the coastal region of Israel (Table 1, Fig. 1). Approximately 10,500 specimens of A. arboreus were collected from 210 nests examined. Up to 4,040 specimens were collected from a single colony. Argas reflexus was found in one poultry house and in feral pigeon nests. By flagging, 245 specimens of Rhipicephalus turanicus, 162 of Rhipicephalus sanguineus and 16 of Hyalomma spec. were collected. Ornithonyssus sylviarum was found in large quantities on laying hens in 6 out of 14 poultry houses examined. Dermanyssus gallinae was found in the nests of the cattle egret, common sparrow (Passer domesticus) and feral pigeon (Columba livia) as well as in poultry houses. Seventy specimens of Astrolaelaps spec. were collected.

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A. arboreus ticks from three out of six cattle egret colonies and 1.6% of the tick pools examined (16 out of 1,000 pools) were positive for WNV by real-time RT-PCR (Table 1). Using the same technique, WNV RNA was identified in one out of 21 O. sylviarum and in one out of 18 D. gallinace pools, while the 63 pools of A. reflexus were negative. Direct RT-PCR was performed with all positive RNA samples but none was positive; therefore, we were unable to determine the nucleotide sequence of any of the positive RNAs. None of the attempts to isolate the virus was successful.

Out of 37 cattle egret squabs examined, 37.8% had neutralizing antibodies to WNV. Titers ranged from 1/10 to 1/640 with more than half the samples being higher than 1/160, indicating a recent infection. Viremia was not detected in any of the blood samples, and virus isolation and direct RT-PCR from their brains were also negative.

Exposure of the arm skin of one of the authors (K.Y.M.) to A. arboreus adults, showed that this tick will feed on humans.

DISCUSSION

In Israel, the cattle egret is a common resident particularly of the northern valleys and the coastal plain. It is found more commonly in boggy habitats, and is frequent in hilly settled areas with fields and trees, and especially around rubbish dumps and sewage ponds. In addition, cattle egret is an uncommon passage migrant and winter visitor in Israel. Lately, it has become a pest in and around villages, settlements and small towns (Shirihai 1997). There have been several complaints regarding damage from their droppings and noise in playgrounds and public gardens, and especially from their ectoparasites such as dermanyssid mites.

<table>
<thead>
<tr>
<th>Geo-climatic region</th>
<th>Host</th>
<th>Parasite</th>
<th>No. pools examined</th>
<th>No. RT-PCR positive pools for WNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal region</td>
<td>B. ibis</td>
<td>A. arboreus</td>
<td>1,000</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>B. ibis</td>
<td>D. gallinace</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>G. gallus</td>
<td>O. sylviarum</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>From the vegetation</td>
<td>R. turanicus</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Galilee mountains (Northern Israel)</td>
<td>G. gallus</td>
<td>O. sylviarum</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Judean and Samaritan range and foothills</td>
<td>C. leucura</td>
<td>A. persicus</td>
<td>56</td>
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</tr>
<tr>
<td></td>
<td>G. gallus</td>
<td>D. gallinace</td>
<td>4</td>
<td>0</td>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>O. sylviarum</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Geo-climatic Areas in Israel from Which Mite and Tick Were Examined for WNV
In the present study, *A. arboreus* was found in large numbers in all the cattle egret colonies. Ticks from three out of six colonies of birds examined were positive for WN viral RNA. To our knowledge, this is the first time that this tick was reported in Israel and the first time that *A. arboreus* collected from the field was WN viral RNA positive. Out of 37 cattle egret squabs examined, 37.8% had serum-neutralizing antibodies to WNV.

Abbassy and others (1993) showed that *A. arboreus* fed artificially on serum containing $10^{-5.3}$ infective doses (IDs) of WNV, reached a peak of $10^{-4}$ (ID) on day 4 post-feeding and remained constant at $10^{-3}$ (ID) for at least 50 days. No evidence of transstadial transmission from second and third nymphs to adults was detected. *A. arboreus* larvae from experimentally infected females successfully transmitted virus to naive chicks and virus was recovered from F1 larvae. It was also found that, in *A. perenius* and in *A. hermanni*, the virus titer declined within a few days to undetectable levels.

Studies on argasid ticks and WNV vectors are of special interest as some of them can also infest humans. *Argas persicus*, *Argas reflexus*, and *Argas latus* are known to infest people worldwide (Theodor and Costa 1967; Mumcuoglu and Rufli 1982; Wilamowski et al. 1999). WNV was isolated from other argasid ticks such as *Argas hermanni* in Africa and Asia (Schmidt and Said, 1964; L’vov 1980; Hoopstraal 1985) and from *A. hermanni*-infested pigeons in Egypt (Schmidt and Said 1964).

Other argasid ticks such as *Ornithodoros savignyi*, *Ornithodoros capensis* and *Ornithodoros erraticus* became infected with WNV by allowing them to feed on infected mice (Hurlbut 1956; Vermeil et al. 1960; Mirzoeva et al. 1974). *O. erraticus* and *Ornithodoros maritimus* were infected by feeding on viremic chickens and rodents and subsequently transmitted the virus to naive hosts (Vermeil et al. 1960). Whitman and Aitken (1960) infected *Ornithodoros moubata* with WNV and demonstrated transmission to susceptible hosts by feeding. Similarly, Lawrie et al. (2004) demonstrated that *O. moubata* maintained infectious virus for at least 132 days, and transmitted the virus transstadially and transovarially as well as to naive hosts.

Regarding hard ticks (Ixodoidea), WNV was isolated from *Hyalomma asiaticum*, *Hyalomma denticatum*, *Hyalomma marginatum*, *R. turanicus*, *Rhipicephalus bursa*, *Ixodes ricinus*, *Amblyomma cajennense* and *Dermacentor marginatus* in Africa and Eurasia (Shalunova et al. 1968; Sokolova et al. 1973; Mirzoeva et al. 1974; Chumakov et al. 1974; L’vov et al. 1975; Gromakeshvisi et al. 1973; Hubalek and Halouzka 1999). WNV was also isolated from a dog tick, *Haemaphysalis leachi* (Blackburn et al. 1990). *Ixodes scapularis*, *Dermacentor andersoni* and *Dermacentor variabilis* infected artificially with
WNV, transmitted it transstadially (Anderson et al. 2003), while *Ixodes ricinus* nymphs infested with WNV upon feeding on viremic host, do not support replication of the virus, and therefore they are not competent vectors (Lawrie et al. 2004). *Rhipicephalus turanicus* is widely distributed in Israel (Mumcuoglu et al. 1993). This species was found on humans, several species of mammals and on birds (Hoogstraal 1956). To our knowledge, this is the first time that this tick was found to be positive for WN viral RNA.

Mesostigmátid mites such as *Dermanyssus gallinae*, *Ornithonyssus sylviarum*, *Haemolaelaps casalis*, and *Haemolaelaps glascowi* are common parasites of wild birds and domestic fowl in Israel and can also infest humans (Theodor and Costa 1967; Mumcuoglu and Rufli 1982). *H. casalis* and the closely related *Haemolaelaps fahrenholzi* can acquire, transmit, and maintain WNV for 30 and 44 days, respectively, and transmit it transovarially and transstadially to their progeny (Tagil-tsev et al. 1977). St. Louis encephalitis virus, and eastern and western equine encephalomyelitis viruses have been isolated from *D. gallinae* collected in nature in Tennessee and Texas (Baker et al. 1967). Western and eastern types of equine encephalomyelitis virus and St. Louis encephalitis virus are also isolated from *O. sylviarum* collected from wild bird nests (Baker et al. 1967). In the present study, 4.8% of the *O. sylviarum* pools collected from laying chickens were WNV RNA positive. The presence of WNV in *D. gallinae* and *O. sylviarum*, which are common parasites of wild birds and domestic fowl in Israel and can infest humans (Theodor and Costa 1967; Mumcuoglu and Rufli 1982). The closely related, *Haemolaelaps casalis* and *Haemolaelaps glascowi* were infected in vitro with WNV and transmitted it to sentinel animals for 30 and 44 days, respectively. The virus was also transmitted transovarially and transstadially to their progeny (Tagil-zev et al. 1977).

The fact that virus isolation was not successful in this study could probably be due to an insufficient amount of virus. The real-time PCR is calibrated to identify as little as 1 PFU, which may or may not be infectious in vitro systems. Ticks were collected during the whole year, that is, also during the period of low bird activity. In addition, viremia may last for few days in birds such as *B. ibis* (Komar et al. 2002).

Our data indicate that several species of ticks and mites may carry WNV. However, additional work is needed to verify whether they are definitive vectors of the virus. One exception is *A. arboreus*, which was demonstrated by Abbasy et al. (1993) to infect naive animals and to transmit the virus transovarially. Though the percentage of infected ticks in the field was low in this study, this is compensated by the very high numbers of ticks found in cattle egret nests. In view of the high percentage of cattle egret nests with antibodies to WNV, we believe that *A. arboreus* can maintain the infection in this bird population throughout the year and that infected birds serve as a source of infection for mosquitoes, thus maintaining the endemic state of WNV in Israel. Since ticks also feed on humans and potentially transmit the virus, it is recommended to remove their nests from the vicinity of human habitations; moreover, egrets are classed as ecological pests in this country.

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