On the potential roles of ticks and migrating birds in the ecology of West Nile virus

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Background: Mosquitoes are the primary vectors of West Nile virus (WNV). Ticks have, however, been suggested to be potential reservoirs of WNV. In order to investigate their role in the spread of the virus, ticks, which had been collected from birds migrating northwards from Africa to Europe, were analyzed for the potential presence of WNV-RNA.

Methods: On the Mediterranean islands Capri and Antikythira a total of 14,824 birds were captured and investigated from which 747 ticks were collected.

Results and conclusions: Most of the identified ticks (93%) were nymphs and larvae of Hyalomma marginatum sensu lato, most of which were or appear to be Hyalomma rufipes. Of these ticks 729 were individually screened for WNV-RNA. None of the ticks was found to be WNV positive. Thus, there was no evidence that Hyalomma marginatum s.l. ticks play a role in the spread of WNV from Africa to Europe.

Keywords: West Nile virus; emerging infectious diseases; migratory birds; zoonoses; ticks; Hyalomma marginatum s.l.; Hyalomma rufipes

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West Nile virus (WNV) belongs to the genus *Flavivirus* of the family *Flaviviridae* (1). It is a mosquito-borne arbovirus with birds as the primary vertebrate host (2). Humans and other mammals are regarded as dead-end hosts. Infection in humans can lead to clinical disease, sometimes with central nervous system complications and high mortality rates, especially in older age groups (3).

WNV infection is considered to be an emerging infectious disease, and there have been numerous epidemics during the last 15 years (4). It is endemic in parts of Africa and epidemic in southern Europe (3). WNV was introduced to the Western Hemisphere as late as 1999 (5). Since then, it has spread over most of North America and has to date (1999–2011) caused more than 1,200 deaths in the United States alone (6). Migratory birds are regarded as the primary means for the virus to spread across the world (7).

Although mosquitoes are the primary vectors, the virus has been isolated repeatedly from both ixodid and argasid ticks, and ticks have been proposed as reservoirs for the virus during bird-associated transfer
of the virus between geographical regions (2, 8, 9). In order to predict and control a potential further geographical spread of this virus, knowledge about its ecology is of utmost importance. To date, not enough data are available to assess the role of ticks in the maintenance and spread of the virus (10). This study aimed to investigate if ticks, which had infested migratory birds in Africa, may be infected with WNV when they arrive on their avian hosts in southern Europe. Thus, at two stopover sites, we net-captured birds that had just crossed the Mediterranean Sea, on their way from their wintering grounds in WNV-endemic Africa to their breeding grounds in Europe. A total of 729 ticks were collected from the birds and individually screened with polymerase chain reaction (PCR) for the presence of WNV RNA.

Materials and methods

Collection of ticks from birds
The birds were captured in mist nets at Capri bird observatory in Italy and at Antikythera bird observatory in Greece in two periods: 2 April–18 May 2009 and 11 March–19 May 2010. Each captured bird was identified to species, and its ears, throat, nape, and abdomen were checked for ticks (11, 12). All ticks observed were removed with forceps, photographed, individually submerged in Eppendorf tubes filled with RNA-later and frozen at −20°C, and stored in RNA-later for 6 months prior to RNA extraction.

RNA extraction and cDNA synthesis
Ticks were homogenized using a Qiagen TissueLyzer (Qiagen) in tubes containing Buffer RLT (Qiagen) with 1% β-mercaptoethanol and a 5 mm steel bead for 2 min at 25 Hz. Each series of RNA extraction also included one NTC and one positive control spiked with Encephalitis virus (TBEV) vaccine (Novartis Vaccines, Basel, Switzerland) and B31 Borrelia spirochetes. After homogenization, RNA extraction was performed in a Qiagen M48 BioRobot using the MagAttract® RNA Tissue Mini M48 kit. The extracted RNA was stored in a −70°C freezer and later used for the WNV screening. Some of the extracted RNA was used for immediate cDNA synthesis. For this, we used a CAS-1200™ Precision Liquid Handling Robot (Corbett Research, Cambridgeshire, UK) to convert RNA into cDNA with the Illustra™ Ready-to-Go RT-PCR beads kit (GE Healthcare, Buckinghamshire, UK). Random hexamer primers pd(N)6 were used to ensure that total RNA was converted. The cDNA was stored in −20°C freezers and then used for the tick species identification.

Tick species identification
The dorsal and ventral sides of each tick were photographed with a Dino-Lite Long 90 × (AM4013TL) USB-microscope (AnMo Electronics Corp., Taiwan). The pictures were analyzed in order to determine the stage and species of the tick. Due to the well-known difficulties in morphological species identification of immature Hyalomma ticks (13, 14), a molecular approach was applied to confirm the identifications based on tick morphology on 10 larval and nymphal tick specimens identified morphologically as Hyalomma sp. and considered to be representative for the entire sample. Available sequences of the different genes of Hyalomma species were compared in GenBank, and the mitochondrial 12S rDNA was used as a target gene.

For the molecular identification of the 10 selected ticks, standard PCR amplifications were carried out with 5 mL of cDNA and 12S rDNA primers (T1B122S and T2A12S) (15).

The PCR products were cloned and subsequently sequenced at the VIB (Flemish Institute for Biotechnology) Genetic Service Facility at the University of Antwerp, using the ABI PRISM BigDye™ Terminator cycle-sequencing kit and an Applied Biosystems 3,730 DNA Analyzer. Sequencing data for the 10 Hyalomma ticks revealed that nine were H. rufipes and one was H. marginatum (16).

WNV screening
A total of 729 ticks were analyzed for the presence of WNV-RNA using a one-step RT qPCR on an ABI 7,900 instrument. Eighteen of the 747 collected ticks were not analyzed due to technical problems. The binding sites of the primers were identified according to Linke et al. (17). These sites were then verified by comparing them with all available WNV sequences in GenBank. A few sequences were eliminated from the alignment due to their geographical origin and considered irrelevant in the present analysis. Degenerate primers were then designed with respect to all remaining sequences (see Table 1). Two separate nucleotide probes were used, one according to a previously described protocol (17), although redesigned as a RGB probe, to detect WNV lineage I and II, as well as a second probe that was designed to detect WNV lineage III, the so-called Rabensburg virus (see Table 1) (18).

| Table 1. Primers and probes for WNV-specific qPCR. |
|---------------------------------|---------------------------------|
| WNV primer fwd. | YCT GYG TGA GCT GAC AAA CTT AGT |
| WNV primer rev. | GCG TTT TWG CAT ATT GAC RGC C |
| WNV probe 1 + 2 | 6-FAM-CCT GGT TTC TTA GAC ATC-MGB |
| WNV probe rab | 6-FAM-ATC AAC AAT TAA TAC AGT GTG AGC-MGB |
|                     | Y = C/T, W = A/T, R = G/A |

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A qScript one-step fast MGB RT-qPCR (Quanta, Rox) kit was used to amplify WNV RNA. Reactions were carried out with a total volume of 20 μL, containing 1.8 μL of each primer (10 μM), 0.2 μL of each probe (5 μM), and 5 μL of template RNA. Extracted RNA from a lineage 1-stem (access no. AF375045) was used as a positive control, and sterile water as a negative control. After an RT-step at 48°C for 5 min and an activation/denaturation step at 95°C for 30 sec, 45 cycles of 95°C for 3 sec and of 60°C for 30 sec were carried out.

Results

A total of 14,824 springtime migratory birds of 78 different species were captured in mist nets at Capri bird observatory and at Antikythira bird observatory in Italy and Greece, respectively (see Table 2). From these birds, we collected 747 ticks (see Table 3). One or more ticks were found on 2.7% of the birds, and the majority of the collected ticks were larvae and nymphs of the Hyalomma marginatum species complex (see Tables 3 and 4). DNA sequencing of the 10 selected Hyalomma ticks was in accordance with the morphological identification (see the ‘Materials and Methods’ section) (16). In total, 29% of the identified ticks were larvae and 70% were nymphs (i.e. 99% of the ticks were immature).

All positive controls from the RNA extractions, spiked with TBEV vaccine and Borrelia, amplified successfully when analyzed (19).

WNV RNA was not detected in any of the PCR reactions performed on the 729 tick samples. However, all negative and positive controls showed adequate curves.

Discussion

WNV infection is considered an emerging infection, and its spread from endemic areas is facilitated by migrating birds (3, 7, 20). It has been shown that long-distance migrating passeriform birds captured in France had a 7% prevalence of WNV-neutralizing antibodies (21). However, a limited time of viremia and an impaired physical condition of WNV-infected birds would presumably reduce their potential to disperse the virus (8, 22, 23). Consequently, as viremic birds may not constitute the only method of viral dispersal from endemic to non-endemic areas, we considered the possibility that hematophagous arthropods infesting migratory birds might contribute to this process. Ticks in WNV endemic areas may attach to migratory birds. Such ticks, possibly acting as reservoirs for WNV and perhaps other pathogens, could then be carried by their avian hosts from WNV-endemic areas in Africa to Europe (8). We have recently shown that this mode of dispersal could play an important role in the spread of another tick-borne pathogen, the Crimean-Congo hemorrhagic fever virus (24).

The birds were caught during rapid northward migration on the small islands where birds normally stop over briefly just after crossing the Sahara Desert and the Mediterranean Sea. Also, most of the collected ticks were either half-fed or fully engorged nymphs, which usually attach to the host already as larvae. This indicates that most or all of these ticks had attached prior to their hosts’ migration (i.e. probably in sub-Saharan and/or North Africa). We therefore speculated that springtime migrating birds, caught at stopover localities in the Mediterranean area, may carry ticks infected with WNV from endemic areas in Africa, and thereafter transfer this potential human pathogen to regions of Southern Europe where outbreaks have recently occurred.

The results from this study do not support the above hypothesis, as none of the analyzed ticks were PCR positive for WNV. Importantly, however, epidemics

| Locality     | 2009 Birds | 2009 Ticks | 2010 Birds | 2010 Ticks | Total Birds | Total Ticks |
|--------------|------------|------------|------------|------------|-------------|-------------|
| Capri        | 4,924      | 251        | 4,022      | 158        | 8,946       | 409         |
| Antikythira  | 2,529      | 135        | 3,349      | 203        | 5,878       | 338         |
| Total        | 7,453      | 386        | 7,371      | 361        | 14,824      | 747         |

Table 2. Number of birds captured and number of ticks collected from the birds.

| Tick genus and species | Number of ticks | Larvae | Nymphs | Adults | Stage unidentifiable |
|-----------------------|-----------------|--------|--------|--------|----------------------|
| Hyalomma marginatum sensu lato | 659          | 195    | 462    | 0      | 2                    |
| Ixodes spp.          | 28             | 7      | 17     | 4**    | 0                    |
| Amblyomma sp.        | 2              | 0      | 2      | 0      | 0                    |
| Haemaphysalis sp.    | 4              | 0      | 4      | 0      | 0                    |
| Genus unidentifiable| 10             | 2      | 6      | 0      | 2                    |
| Total                | 703*           | 204    | 491    | 4      | 4                    |

*44 ticks could not be identified to genus, species, or stage due to missing photos or unidentifiable condition of the specimens. **Ixodes frontalis.
of WNV have been reported recently from locations close to Antikythera, Greece, and Capri, Italy. In 2008–2009, about 20 people contracted West Nile neuroinvasive disease (WNND) in and around the Veneto region of northeastern Italy, and in 2010 the epidemic had spread to other regions of Italy, namely Sicily and Molise, both of which are close to the island of Capri (25, 26). In 2010, an outbreak of 81 cases of WNND was reported in central Macedonia in northwestern Greece (27). Thus, the two locations chosen for the collection of possible arthropod vectors for WNV are highly relevant since they are situated on birds’ migration routes between Africa and WNV epidemic areas in Europe.

Most of the collected ticks were larvae and nymphs that appeared to belong to *H. rufipes* in the *H. marginatum* complex. These results are similar to those of Hoogstraal et al. (28) since, in both investigations, nearly all ticks were identified as immatures that appeared to be *H. rufipes* (28).

**Table 4. Bird species infested with ticks.**

| Scientific name                 | Common name                     | Number of birds | Number of ticks | Number of (%) birds infested | Mean infestation rate (Number of ticks/number of infested birds) |
|---------------------------------|---------------------------------|-----------------|-----------------|-----------------------------|------------------------------------------------------------------|
| *Acrocephalus arundinaceus*     | Great reed warbler              | 113             | 11              | 3 (2.7)                     | 3.7                                                              |
| *Acrocephalus schoenobaenus*    | Sedge warbler                   | 452             | 39              | 13 (2.9)                    | 3.0                                                              |
| *Acrocephalus scirpaceus*       | European reed warbler           | 23              | 6               | 2 (8.7)                     | 3.0                                                              |
| *Anthus trivialis*              | Tree pipit                      | 409             | 16              | 12 (2.9)                    | 1.3                                                              |
| *Caprimulgus europaeus*         | European nightjar               | 17              | 2               | 1 (5.9)                     | 2.0                                                              |
| *Carduelis chloris*             | European greenfinch             | 11              | 4               | 1 (9.1)                     | 4.0                                                              |
| *Carduelis spinus*              | Eurasian siskin                 | 3               | 1               | 1 (33)                      | 1.0                                                              |
| *Erithacus rubecula*            | European robin                  | 133             | 18              | 10 (7.5)                    | 1.8                                                              |
| *Ficedula albicollis*           | Collared flycatcher             | 160             | 6               | 3 (1.9)                     | 2.0                                                              |
| *Ficedula hypoleuca*            | Pied flycatcher                 | 2,013           | 95              | 63 (3.1)                    | 1.5                                                              |
| *Hippolais icterina*            | Icterine warbler                | 476             | 7               | 6 (1.3)                     | 1.2                                                              |
| *Hippolais pallida*             | Eastern olivaceous warbler      | 46              | 2               | 2 (4.3)                     | 1.0                                                              |
| *Lanius senator*                | Woodchat shrike                 | 144             | 51              | 18 (13)                     | 2.8                                                              |
| *Luscinia megarhynchos*         | Nightingale                     | 320             | 35              | 13 (4.1)                    | 2.7                                                              |
| *Motacilla flava*               | Yellow wagtail                  | 10              | 11              | 2 (20)                      | 5.5                                                              |
| *Muscicapa striata*             | Spotted flycatcher              | 1,039           | 7               | 7 (0.7)                     | 1.0                                                              |
| *Oenanthe oenanthe*             | Wheatear                        | 53              | 21              | 8 (15)                      | 2.6                                                              |
| *Oriolus oriolus*               | Eurasian golden oriole          | 295             | 19              | 13 (4.4)                    | 1.5                                                              |
| *Phoenicurus phoenicurus*       | Common redstart                 | 383             | 55              | 31 (8.1)                    | 1.8                                                              |
| *Phylloscopus orientalis*       | Eastern Bonelli’s warbler       | 26              | 7               | 4 (15)                      | 1.8                                                              |
| *Phylloscopus sibilatrix*       | Wood warbler                    | 1,239           | 33              | 30 (2.4)                    | 1.1                                                              |
| *Phylloscopus trochilus*        | Willow warbler                  | 738             | 4               | 4 (0.5)                     | 1.0                                                              |
| *Saxicola rubetra*              | Whinchat                        | 1,476           | 141             | 74 (5.0)                    | 1.9                                                              |
| *Sylvia borin*                  | Garden warbler                  | 2,191           | 13              | 11 (0.5)                    | 1.2                                                              |
| *Sylvia communis*               | Common                          | 1,245           | 122             | 68 (5.5)                    | 1.8                                                              |
| *Turdus philomelos*             | Song thrush                     | 22              | 18              | 3 (14)                      | 6.0                                                              |
| *Upupa epops*                   | Hoopoe                          | 10              | 2               | 2 (20)                      | 1.0                                                              |
| Other species                   |                                 | 1,777           | 1               | 1 (0.1)                     |                                                                 |
| Total                           |                                 | 14,824          | 747             | 406 (2.7)                   | 1.8                                                              |

*One tick was found on an unidentified bird species.*
The tick infestation rate of 2.7% that was recorded in this study is also similar to that of Hoogstraal et al. (28). They found a tick infestation rate of 3.0% on birds captured in Egypt during their spring migration from East Africa to Europe.

Moskvitina et al. (29) detected WNV RNA and WNV antigen in *Ixodes pavlovskyi* and *Ixodes persulcatus*. The WNV-positive ticks had been collected from small mammals, lizards, and birds in the Tomsk Region, Russian Siberia (29).

Laboratory experiments have revealed that *H. marginatum* became infected with WNV after a blood meal from viremic hosts. The infection rates of the larval, nymphal, and adult ticks were 3, 33 and 75%, respectively. Both transstadial infection and the capacity of nymphal and adult ticks to transmit the virus to previously uninfected hosts were demonstrated (30). In Israel, ticks were collected from wild and domesticated birds and their nests, and analyzed for the presence of WNV. A total of 1.6% of *Argas arboresus* pools were positive, but none of the *Hyalomma* ticks. This is in agreement with our study. The authors suggested that some tick species may play a role in maintaining the infection in Israel (9). Reisen and coworkers (31) investigated the ability of transstadially infected *Ixodes pacificus* to transmit WNV to song sparrows and western fence lizards (31). Based on their results and previous studies, these scientists concluded that there are indications that ixodid ticks are not able to experimentally transmit WNV and therefore most likely would not be important vectors in WNV transmission cycles.

Our results do not support the hypothesis that *Hyalomma* ticks play a major role as a WNV reservoir on their avian hosts’ northward flight from Africa to Europe. The information so far obtained regarding the potential role of ticks as reservoirs and vectors is inconclusive. Further laboratory experiments on the reservoir and vector competency of different tick species are needed. Also, investigations based on a larger number of ticks of different species and geographic origins are needed to better understand the potential role of ticks in the ecology of the WNV.

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Conflict of interest and funding

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References

1. Calisher CH, Karabatsos N, Dalrymple JM, Shope RE, Porterfield JS, Westaway EG, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 1989; 70: 37–43.
2. Hubálek Z, Halouzka J. West Nile fever – a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis 1999; 5: 643–50.
3. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. Lancet Infect Dis 2002; 9: 519–29.
4. Zeller HG, Schuffenecker I. West Nile virus: an overview of its spread in Europe and the Mediterranean basin in contrast to its spread in the Americas. Eur J Clin Microbiol Infect Dis 2004; 3: 147–56.
5. Nash D, Mostashari F, Fine A, Miller J, O’Leary D, Murray K, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med 2001; 24: 1807–14.
6. CDC. West Nile virus – statistics, surveillance, and control > Case Count 2010. Available from: http://www.cdc.gov/ncidod/dvbd/westnile/surv&controlCaseCount10.detailed.htm [cited 11 January 2011].
7. Rappole JH, Derrickson SR, Hubálek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. Emerg Infect Dis 2000; 4: 319–28.
8. Reiter P. West Nile virus in Europe: understanding the present to gauge the future. Euro Surveill 2010; 15: 19508.
9. Muncuoglu KY, Banet-Noach C, Malkinson M, Shalom U, Galun R. Argasid ticks as possible vectors of West Nile virus in Israel. Vector Borne Zoonotic Dis 2005; 1: 65–71.
10. Granwehr BP, Lillibridge KM, Higgins PW, Mason PW, Aronson JF, Campbell GA, et al. West Nile virus: where are we now? Lancet Infect Dis 2004; 9: 547–56.
11. Svensson L. Identification guide to European passerines. Stockholm: Fingraf; 1992.
12. Baker K. Identification guide to European non-passerines. London: British Trust for Ornithology, 1993.
13. Apanaskevich DA, Horak IG. The genus Hyalomma Koch, 1844: v. re-evaluation of the taxonomic rank of taxa comprising the *H. (Euhyalomma)* marginatum Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology. Int J Acarol 2008; 1: 13–42.
14. Apanaskevich DA, Santos-Silva MM, Horak IG. The genus Hyalomma Koch, 1844. IV. Redescription of all parasitic stages of *H. (Euhyalomma)* lusitanicum Koch, 1844 and the adults of *H. (E.) franchimii Tonelli Rondelli, 1932 (acari: ixodidae) with a first description of its immature stages. Folia Parasitol 2008; 1: 61–74.
15. Beati L, Keirans JE. Analysis of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J Parasitol 2001; 1: 32–48.
16. Molin Y, Lindeborg M, Nystöm F, Madder M, Hjelm E, Olsen B, et al. Migratory birds, ticks, and Bartonella. Infect Ecol Epidemiol 2011; 1: 3597.
17. Linke S, Ellerbrok H, Niedrig M, Nitsche A, Pauli G. Detection of tick borne encephalitis virus (TBEV) RNA. J Clin Virol 2003; 2: 136–45.
20. Dusek RJ, McLean RG, Kramer LD, Ubico SR, Dupuis AP, 2nd, Ebel GD, et al. Prevalence of West Nile virus in migratory birds during spring and fall migration. Am J Trop Med Hyg 2009; 6: 1151–8.
21. Jourdain E, Zeller HG, Sabatier P, Murri S, Kayser Y, Greenland T, et al. Prevalence of West Nile virus neutralizing antibodies in wild birds from the Camargue area, southern France. J Wildl Dis 2008; 3: 766–71.
22. McLean RG, Ubico SR, Docherty DE, Hansen WR, Sileo L, McNamara TS. West Nile virus transmission and ecology in birds. Ann N Y Acad Sci 2001; 951: 54–7.
23. Shirafuji H, Kanehira K, Kubo M, Shibahara T, Kamio T. Experimental West Nile virus infection in jungle crows (Corvus macrorhynchos). Am J Trop Med Hyg 2008; 5: 838–42.
24. Lindeborg M, Barboutsis C, Ehrenborg C, Fransson T, Jaenson TGT, Lindgren P-E, et al. Migratory birds, ticks, and Crimean-Congo hemorrhagic fever virus. Emerg Infect Dis 2012; 12: 2095–7.
25. Rizzo C, Vescio F, De Clech S, Finarelli AC, Macini P, Mattivi A, et al. West Nile virus transmission with human cases in Italy, August–September 2009. Euro Surveill 2009; 14: 19353.
26. Calistri P, Monaco F, Savini G, Guercio A, Purpari G, Vicari D, et al. Further spread of West Nile virus in Italy. Vet Ital 2010; 4: 467–74.
27. Papa A, Danis K, Baka A, Bakas A, Douglas G, Lytras T, et al. Ongoing outbreak of West Nile virus infections in humans in Greece, July–August 2010. Euro Surveill 2010; 15: 19644.
28. Hoogstraal H, Kaiser MN, Traylor MA, Gaber S, Guindy E. Ticks (Ixodoidea) on birds migrating from Africa to Europe and Asia. Bull World Health Organ 1961; 24: 197–212.
29. Moskvitina NS, Romanenko VN, Ternovoi VA, Ivanova NV, Protopopova EV, Kravchenko LB, et al. [Detection of the West Nile Virus and its genetic typing in ixodid ticks (Parasitiformes: Ixodidae) in Tomsk City and its suburbs]. Parazitologija 2008; 3: 210–25.
30. Formosinho P, Santos-Silva MM. Experimental infection of Hyalomma marginatum ticks with West Nile virus. Acta Virol 2006; 3: 175–80.
31. Reisen WK, Braught AC, Martinez VM, Fang Y, Simmons K, Garcia S, et al. Ability of transstadially infected Ixodes pacificus (Acarci: Ixodiidae) to transmit West Nile virus to song sparrows or western fence lizards. J Med Entomol 2007; 2: 320–7.

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