Seroprevalence of West Nile virus, Crimean-Congo hemorrhagic fever virus, *Francisella tularensis* and *Borrelia burgdorferi* in rural population of Manisa, western Turkey

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ABSTRACT

*Background & objectives:* Zoonotic diseases are well recognised threat to public health globally. The information of regional prevalence and associated risk factors allow the national programmes to determine and frame better strategies for their control, as they also provide the actual status of zoonosis in the region. The aim of this study was to determine the seroprevalence of West Nile virus (WNV), Crimean-Congo hemorrhagic fever virus (CCHFV), *Francisella tularensis* and *Borrelia burgdorferi* among the rural residents of Manisa region, Turkey and to identify the associated risk factors.

*Methods:* Cross sectional study was conducted in rural parts of Manisa, Aegean region of western Turkey in 2012. Blood samples from 324 randomly selected subjects were screened for the presence of IgG antibodies to WNV, CCHFV, *F. tularensis* and *B. burgdorferi* with commercially available kits. The demographic structure of the rural residents and risk factors related to lifestyle such as outdoor agriculture activities, animal husbandry, hunting and history of tick bite were questioned and their relationships with positive results were analyzed statistically.

*Results:* It was observed that 49 subjects (15%) had IgG antibodies to at least one of the zoonotic agents studied. The seroprevalence of *F. tularensis* was highest with a percentage of 7.1% (n = 23). Distribution of the positive results for WNV, CCHFV and *B. burgdorferi* were 4.3% (n = 14), 3.7% (n = 12) and 0.9% (n = 3), respectively. Older age and uncompleted secondary education were the statistically significant risk factors for seropositivity to at least one zoonotic agent investigated. Logistic regression analyses confirmed that older age (over 50) increased the risk of WNV and CCHFV seroposivity.

*Interpretation & conclusion:* Seropositivity rates were not found to be higher than the expected rates. Further, studies are needed to evaluate the threat of vector borne zoonoses and associated risk factors in the study area.

Key words  *Borrelia burgdorferi*; CCHFV; *Francisella tularensis*; rural area; seroprevalence; WNV

INTRODUCTION

In recent years, similar to other parts of the world, a variety of vector borne zoonoses, either emerging or re-emerging, have gained considerable attention in Turkey. Out of these, the tularemia, lyme disease, West Nile virus (WNV) and Crimean-Congo haemorrhagic fever virus (CCHFV) infections have become particularly important as they are endemic in many rural regions of the country, and sporadic cases or outbreaks have been reported almost every year¹-⁶.

Moreover, WNV is a zoonosis of greater interest for the Manisa province, since it first came to light in 2010 along with the encephalitis cases reported in the province⁸. Following the unprecedented increase in the number of WNV cases, a surveillance study was conducted in Turkey, from July to November 2010; and at the same time, a serosurvey was also carried out in three provinces having high incidence of WNV cases. The study revealed that 4 of 104 (3.8%) serum samples obtained from Manisa, and 13 of 40 (32.5%) and 15 of 69 (21.7%) from Mugla (Aegean region) and Sakarya (western Turkey), respectively, were positive for WNV antibodies. In the same study, taking into consideration the distribution of detected human WNV cases in Turkey (n = 47), authors concluded that WNV was widespread in western part of the country except Diyarbakir (a province in the southeast of Turkey)⁷.

However, in a serological, molecular and entomological surveillance study conducted in the following years (2011–13), it was reported that WNV activity was evident in a large area encompassing the southeast and northeast Anatolian as well as Mediterranean-Aegean and eastern Thrace regions of Turkey⁸.
In Turkey, another zoonosis having an increasing impact on the rural residents is tularemia which is a vector borne or water borne disease. Despite many years of evidence on the presence of the disease, tularemia cases in the country showed a significant increase starting from 2009 with 428 reported cases and reached 1,531, 2,151, 6,07 in 2010, 2011, and 2012, respectively. Sporadic cases and outbreaks mainly linked to consumption of contaminated natural spring waters have been reported in many regions of Turkey but mainly in Black Sea, Marmara and Thrace regions.

Unlike the other diseases investigated in this study, Lyme borreliosis is not a notifiable disease in Turkey, and despite the abundance of reported cases in the Turkish literature the true prevalence of the disease in the populations under risk is still not known. To the best of our knowledge there is no study on the prevalence of these zoonoses in the human population of Manisa region and the prevalence of these diseases and their association with recognized risk factors still remains unknown.

The main objective of this preliminary study was to determine the seroprevalence of IgG antibodies to WNV, CCHFV, *B. burgdorferi* and *F. tularensis* in apparently healthy population living in the rural areas of Manisa, western Turkey, and to investigate whether any relationship existed between serostatus and the related risk factors.

**MATERIAL & METHODS**

**Study area**

Manisa is a province in the Aegean region of western Turkey (38°36'51.0"N; 27°26'03.0"E). The seat of province is the City of Manisa, which is the one of the largest industrial centers of Turkey. The total population of the province is 1,346,162 of which 441,649 are the rural residents living in 780 villages of 16 districts.

**Study design**

As a representative sample, 450 rural residents were randomly selected from 10 of the 20 villages located at least 10 km away from a district centre, with population over 1000, and where farming was the main source of income. Sample size was calculated using Epi Info software, taking the expected prevalence as 10% (confidence limits as ± percent of 100 as 4), and desired level of confidence as 95%. Randomly selected sample list (stratified by age and sex) was obtained from Manisa provincial directorate of public health’s database of family medicine information system.

**Data collection**

Interviews with 324 of the 450 selected people were carried out between September and December 2012. After obtaining written informed consent, subjects were asked to fill in a questionnaire including data about sociodemographics, outdoor agricultural activities, animal husbandry, and other activities or factors related to increased risk of zoonotic infections. In order to detect whether there was a relation between *F. tularensis* seropositivity and consumption of non-chlorinated water from natural sources, information on consumption of non-chlorinated water was gathered.

**Sample collection**

The blood samples from individuals aged 18 yr and older were obtained from the selected villages of the following districts: 84 from Akhisar, 82 from Sarigöl, 36 from Salihli, 35 from Alasehir, 32 from Demirci, 28 from Manisa, 27 from Kula (Fig. 1). All the subjects included in the study were healthy and had no signs of infection.

**Serological assays**

Enzyme linked immunosorbent assay (ELISA) was performed for the detection of IgG antibodies against WNV (Euroimmun, Germany), and *F. tularensis* (Virion/Serion, Germany). Criteria for the interpretation of ELISA serologic analyses and the diagnostic specificity and sensitivity according to manufacturer’s guidelines are given in Table 1. Commercially available indirect immunofluores-
IgG antibody detection was performed using indirect immunofluorescence assay (IFA) kits according to the manufacturer’s guidelines (Euroimmun, Germany). The sensitivity and specificity of the IFA tests for CCHF virus Mosaic 2 IgG, B. burgdorferi IgG (VisE) and WNV IgG according to manufacturer’s guidelines were 89.5, 95 and 100%; and 100, 93 and 98% respectively.

The sera positivity for B. burgdorferi with IIFA test was confirmed by western blot (WB) method (Euroimmun, Germany). The sera considered as positive for WNV IgG by ELISA were further tested by IIFA (Euroimmun, Germany). Equivocal (grey zone or borderline) results obtained with the serological assays were grouped with the negative results.

Statistical analyses

A statistical software (SPSS 15.0) was used for all the analyses. The association between investigated risk factors and seropositivity was analyzed using logistic regression model. The odds ratios (ORs) with 95% confidence intervals were calculated for determining the relation of the seroprevalence to zoonoses and risk factors. \( P \leq 0.05 \) was considered as statistically significant.

Ethical approval

The study protocol was approved by the Clinical Research Ethics Committee (Dokuz Elyul University, 09. 08.2012; No: 2012/17-06).

RESULTS

Out of the participants, 48.1% were male and 51.9% were female. Mean age of the study group was 49.16 ± 16.78. Subjects with unfinished secondary education (83%) comprised the majority of the study group while 71.6% were farmers, 94.4 and 56.8% of subjects were engaged in agriculture activities and animal husbandry, respectively.

Out of 324 study subject 49 (15%) were found to carry IgG antibodies to at least one of the zoonotic agents investigated. Estimated seroprevalence for F. tularensis, WNV and CCHFV were 7.1% (n = 23), 4.3% (n = 14) and 3.7% (n = 12), respectively. Borrelia burgdorferi IgG seropositivity rate confirmed by WB was 0.9% (n = 3). All WNV IgG positive samples showed positive results by both ELISA and IIFA.

Although seroprevalence rates for F. tularensis were more among females, according to sex statistically significant association was not found for any zoonosis investigated. However, older age and uncompleted secondary education were the statistically significant risk factors for seropositivity to at least one zoonotic agent investigated. Regression analysis revealed that age over 50 yr was associated with increasing odds of having IgG seropositivity to CCHFV and WNV. The other investigated risk factors had no significant influence on the serostatus of rural residents (Table 2).

DISCUSSION

The prevalence of zoonoses varies according to the geographical area, as well as the socioeconomic and sociocultural status of the residents. For this reason, determining the regional prevalence and associated risks are extremely important. The epidemiological studies conducted in Turkey to estimate the prevalence of CCHFV have reported that the Ig G seropositivity rates varied from 0–19.6% with the highest rates being in endemic areas and in those living in rural areas, engaged in animal husbandry and exposed to tick bite12–14.

In a recent study conducted in seven provinces representing seven geographical regions of Turkey, the reported overall seroprevalence was 2.3%. While the mean rate in the rural areas was 4.1%, a high seropositivity rate of 16.7% was detected in the case reported areas. In the same study, no seropositive subjects were detected in the urban areas of Aydin representing the Aegean region of Turkey, where Manisa is also located; while the rate of seropositivity was 4.1% in the rural areas14.

CCHFV IgG positivity which was found to be 3.7% in our study is consistent with the rural data of Turkey as well as the data recently reported from Greece (3.4%), Bulgaria (3.2%) and Kosovo (4.0%)15–17. Although, the seropositivity was found to be not associated with risk factors investigated, its rate was high in the subjects over ≥50 yr-old, which indicates that the causative agent is persisting in the area and the older residents are at higher risk. Moreover, the protective clothing used by the resi-

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**Table 1. Criteria for the interpretation of ELISA tests and their diagnostic specificity and sensitivity**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Positive</th>
<th>Grey zone</th>
<th>Negative</th>
<th>% Specificity</th>
<th>% Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Nile virus</td>
<td>≥ 22 RU/ml</td>
<td>≥ 16 – &lt;22 RU/ml</td>
<td>&lt;16 RU/ml</td>
<td>96.9</td>
<td>99.5</td>
</tr>
<tr>
<td>Francisella tularensis</td>
<td>&gt; 15 U</td>
<td>10 – 15 U</td>
<td>&lt;10 U</td>
<td>96.9</td>
<td>&gt; 99</td>
</tr>
</tbody>
</table>

RU—Relative unit; U—Unit.
Table 2. Odds ratio estimates for seroprevalence of zoonoses by risk factor calculated with logistic regression

<table>
<thead>
<tr>
<th>Variables</th>
<th>CCHFV (n = 12)</th>
<th>WNV (n = 14)</th>
<th>Francisella tularensis (n = 23)</th>
<th>Borrelia burgdorferi (n = 3)</th>
<th>Seropositive to at least one zoonotic agent (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% OR (95% CI)†</td>
<td>% OR (95% CI)†</td>
<td>% OR (95% CI)†</td>
<td>% OR (95% CI)†</td>
<td>% OR (95% CI)†</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 (n = 172)</td>
<td>1.7 0.27 (0.07–1.04)*#</td>
<td>1.7 0.22 (0.06–0.81)*#</td>
<td>6.4 Ref</td>
<td>0.6 0.41 (0.03–4.62)*#</td>
<td>10.5 0.45 (0.24– 0.85)*#</td>
</tr>
<tr>
<td>≥50 (n = 152)</td>
<td>5.9 Ref</td>
<td>7.2 Ref</td>
<td>7.9 0.81 (0.34–1.90)†</td>
<td>1.3 Ref</td>
<td>20.4 Ref</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n = 168)</td>
<td>3 Ref</td>
<td>3 Ref</td>
<td>9.5 Ref</td>
<td>0 Ref</td>
<td>14.3 Ref</td>
</tr>
<tr>
<td>Male (n = 156)</td>
<td>4.5 1.59 (0.49–5.15)</td>
<td>5.8 2.09 (0.68–6.47)</td>
<td>4.5 0.44 (0.17–1.12)</td>
<td>1.9 NA</td>
<td>16 1.17 (0.63–2.16)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Farmer (n = 232)</td>
<td>3.9 1.45 (0.37–5.59)</td>
<td>4.3 1.23 (0.36–4.09)</td>
<td>8.2 2.06 (0.67–6.31)</td>
<td>0.9 0.66 (0.05–7.49)</td>
<td>16.4 1.66 (0.80–3.47)</td>
</tr>
<tr>
<td>Other (n = 92)</td>
<td>3.3 Ref</td>
<td>4.3 Ref</td>
<td>4.3 Ref</td>
<td>1.1 Ref</td>
<td>12 Ref</td>
</tr>
<tr>
<td>Secondary education</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes (n = 55)</td>
<td>1.8 Ref</td>
<td>0 Ref</td>
<td>1.8 Ref</td>
<td>0 Ref</td>
<td>3.6 Ref</td>
</tr>
<tr>
<td>No (n = 269)</td>
<td>4.1 1.22 (0.13–11.22)</td>
<td>5.2 NA</td>
<td>8.2 4.85 (0.61–38.24)</td>
<td>1.1 NA</td>
<td>17.5 4.34 (0.98–19.11)*#</td>
</tr>
<tr>
<td>Outdoor agricultural activities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 306)</td>
<td>3.3 0.40 (0.07–2.08)</td>
<td>3.6 0.28 (0.07–1.19)</td>
<td>7.2 1.47 (0.18–11.92)</td>
<td>1 NA</td>
<td>14.4 0.58 (0.19–1.76)</td>
</tr>
<tr>
<td>No (n = 18)</td>
<td>11.1 Ref</td>
<td>16.7 Ref</td>
<td>5.6 Ref</td>
<td>0 Ref</td>
<td>27.8 Ref</td>
</tr>
<tr>
<td>Animal husbandry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 184)</td>
<td>4.3 1.71 (0.49–5.86)</td>
<td>3.3 0.61 (0.20–1.89)</td>
<td>7.1 1 (0.42–2.37)</td>
<td>0.5 0.45 (0.04–5.13)</td>
<td>14.1 0.88 (0.47–1.64)</td>
</tr>
<tr>
<td>No (n = 140)</td>
<td>2.9 Ref</td>
<td>5.7 Ref</td>
<td>7.1 Ref</td>
<td>1.4 Ref</td>
<td>16.4 Ref</td>
</tr>
<tr>
<td>Hunting</td>
<td></td>
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</tr>
<tr>
<td>Yes (n = 29)</td>
<td>0 NA</td>
<td>0 NA</td>
<td>3.4 0.46 (0.05–3.57)</td>
<td>3.4 2.21 (0.19–25.28)</td>
<td>6.9 0.45 (0.10–2)</td>
</tr>
<tr>
<td>No (n = 294)</td>
<td>4.1 Ref</td>
<td>4.8 Ref</td>
<td>7.5 Ref</td>
<td>0.7 Ref</td>
<td>16 Ref</td>
</tr>
<tr>
<td>History of tick bite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 13 )</td>
<td>7.7 1.94 (0.22–16.67)</td>
<td>7.7 1.60 (0.18–13.59)</td>
<td>7.7 1.05 (0.13–8.55)</td>
<td>0 NA</td>
<td>23.1 1.55 (0.40–5.97)</td>
</tr>
<tr>
<td>No (n = 311)</td>
<td>3.5 Ref</td>
<td>4.2 Ref</td>
<td>7.1 Ref</td>
<td>1 Ref</td>
<td>14.8 Ref</td>
</tr>
<tr>
<td>Application of tick control products on animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 168)</td>
<td>3.6 Ref</td>
<td>3 Ref</td>
<td>7.1 Ref</td>
<td>0.6 Ref</td>
<td>13.1 Ref</td>
</tr>
<tr>
<td>No (n = 65)</td>
<td>1.5 0.40 (0.04–3.45)</td>
<td>1.5 0.48 (0.05–4.24)</td>
<td>10.8 1.55 (0.58–4.14)</td>
<td>0 NA</td>
<td>13.8 1.04 (0.45–2.42)</td>
</tr>
<tr>
<td>Protective clothing against tick</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 231)</td>
<td>3 Ref</td>
<td>4.8 Ref</td>
<td>7.8 Ref</td>
<td>0 Ref</td>
<td>14.7 Ref</td>
</tr>
<tr>
<td>No (n = 91)</td>
<td>5.5 2.04 (0.62–6.70)</td>
<td>3.3 0.74 (0.20–2.76)</td>
<td>5.5 0.69 (0.25–1.94)</td>
<td>3.3 NA</td>
<td>16.5 1.21 (0.61–2.37)</td>
</tr>
</tbody>
</table>

CCHFV—Crimean-Congo hemorrhagic fever virus; WNV—West Nile virus; OR—Odd ratio; CI—Confidence interval; Ref—Reference; NA—Not applicable; *Adjusted for age except for age variables; *p < 0.05; #Adjusted for sex; Missing data: 1(n = 2), 2(n = 91), 3(n = 2).
dents and application of tick control products on animals were found insufficient, therefore they were informed about tick control activities for preventing the disease.

In Turkey, the WNV seropositivity rates, as confirmed by plaque reduction neutralization test (PRNT), which is the gold standard serological test, have been reported to be as low as 0.8% in blood donors, and as high as 17% in human population at risk\textsuperscript{18–19}. Even though the WNV seroprevalence rate obtained in this study appears comparable to that reported in an earlier study from Manisa in 2010 (3.8%), and to some extent with those recently reported from healthy blood donors of western Turkey (2.5%) which were also obtained with ELISA and IIFA, it should be kept in mind that the positivity rate, which was 4.3% could be reflecting the past exposures or could be a non-specific reactivity as we were unable to confirm the results with PRNT\textsuperscript{7, 20}. The results of the present study are preliminary findings which highlight the current situation in the Manisa region and could be used in the future studies.

On the other hand, identification of \textit{Culex pipiens}, the vector of WNV, in a previous study conducted to identify the mosquito species and the potential of mosquito related diseases in Manisa province has led to a consideration that WNV should still be regarded as a threat in the region\textsuperscript{21}. Moreover, given the evidences of WNV circulation in the Mediterranean countries, further studies employing reference method and including both the mammalian hosts and the mosquitoes are required to obtain data reflecting the WNV epidemiology of the Aegean region in a better way\textsuperscript{22}.

The observed prevalence for tularemia in this study was 7.1%. Even though we didn’t find any significant association between seropositivity and the variables studied, taking into consideration the high frequency of water borne tularemia cases in Turkey, it was thought that the risky behaviour such as drinking untreated water (30%) from natural resources among the rural residents should be avoided\textsuperscript{23–24}. Also, more comprehensive studies should be performed to explain modes of transmission of this zoonotic agent to humans in Turkey.

Although, the seropositivity rates of \textit{B. burgdorferi} (mainly unconfirmed by WB) in high risk groups were reported between 6% and 44% in the earlier Turkish studies, in a recent study conducted in the Black Sea region of Turkey with a high population of ticks, 3.3% of the 419 healthy study subjects were seropositive for \textit{B. burgdorferi} by WB\textsuperscript{10, 25}. Similarly, Kaya AE \textit{et al}\textsuperscript{26} have reported that the seroprevalence of \textit{B. burgdorferi} among forestry workers and farmers was 1.1% (confirmed by WB) and the infections caused by this agent were not common in Duzce, northwestern Turkey. However, in both of these studies, the authors emphasized that the seropositivity for \textit{B. burgdorferi} was higher in those living in the rural areas and the seropositivity was associated with tick bite history and contact with animals. No similar association was found in our study, but the seroprevalence rate obtained (0.9%) was not much different from those reported earlier, which indicates that this zoonosis should not be considered as a major threat in the region. Also, the present result was in agreement with the previously reported low seroprevalence rate in a Greek adult population (0.27%)\textsuperscript{27}. However, prevalence rates higher than those observed in this study have been reported from some European countries, such as Norway (6.4%) and Germany (9.4%)\textsuperscript{28–29}.

**CONCLUSION**

In conclusion, despite some limitations of this study (measuring IgG only and not being able to perform all relevant supplementary tests); identifying the spatial distribution of zoonoses is important, since it would contribute to regional mapping of the zoonoses, which may shed light on regional variability. This, in turn, can be used to develop and plan control strategies at smaller scale that would enable better allocation of resources within the region and protect the public health.

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