Serological investigation for West Nile virus, Anaplasma ovis and Leishmania infantum in Greek cattle

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Introduction

Cattle are regarded as a natural reservoir for some infectious diseases affecting livestock as well as humans (Muma et al. 2010). Infectious agents such as Anaplasma spp. (Aktas et al. 2011) and Leishmania infantum ( Alam et al. 2011) can be found in cattle, which consequently can be regarded as potential reservoirs for leishmaniosis (at least in other parts of the world such as Asia) (Singh et al. 2013) and anaplasmosis (de la Fuente et al. 2008). In the case of WNV, cattle may serve as sentinel of the presence of the pathogen in an area.

Keywords
Anaplasma ovis, Cattle, Greek, Leishmania infantum, Seroprevalence, West Nile virus, Zoonoses.

Summary

This study investigates the seroprevalence of Greek adult cattle against West Nile virus, Anaplasma ovis and Leishmania infantum. In total, 156 serum samples were examined, drawn from cattle between 2-4 years old. All the examined cattle originated from slaughterhouses of 4 prefectures in Northern Greece (Thessaloniki, Pella, Chalkidiki, Kilkis), in 2 of which (Thessaloniki, Pella) human cases of West Nile virus had been recorded some months before. Thirty out of the 156 (18.6%) samples have tested positive for West Nile virus and fifty-five (35.9%) samples for Anaplasma ovis. All the examined samples tested negative for Leishmania infantum. The prefectures with positive samples against West Nile virus also showed human cases of West Nile virus infections. This should raise questions whether cattle could become markers for West Nile virus activity in high risk areas.

Parole chiave
Anaplasma ovis, Bovino, Grecia, Leishmania infantum, Sieroprevalenza, Virus della West Nile, Zoonosi.

Riassunto

Lo studio prende in esame le sieroprevalenze del virus della West Nile, di Anaplasma ovis e di Leishmania infantum in bovini da 2 a 4 anni di età nel Nord della Grecia. Tra luglio e settembre 2010 sono stati prelevati 156 campioni provenienti da macelli di 4 prefetture. In 2 di queste (Thessaloniki, Pella) erano stati riportati, precedentemente a questa indagine, casi di West Nile nell’uomo. Trenta (18,6%) dei campioni analizzati hanno risultato positivi al virus della West Nile e 55 (35,9%) ad Anaplasma ovis. Tutti i 156 campioni sono risultati negativi a Leishmania infantum. Le prefectures with positive samples against West Nile virus also showed human cases of West Nile virus infections. This should raise questions whether cattle could become markers for West Nile virus activity in high risk areas.
To date, the epidemiological status of these infections in cattle in Greece is unknown, although these agents or their antibodies have been recorded in other animal species or humans. West Nile infection has been recorded in humans, chickens and wild birds (Chaskopoulou et al. 2011, Papa et al. 2011a, Papa et al. 2011b, Valiakos et al. 2011), and during 2010, human outbreaks with fatalities occurred (Chaskopoulou et al. 2011, Papa et al. 2011a). Anaplasma ovis has been found to cause disease in small ruminants in Greece (Giadinis et al., 2011), and possible cases in humans have also been reported (Chochlakis et al. 2010), at the same time Anaplasma phagocytophilum has been detected in Ixodes ricinus in Northern Greece (Kachrimanidou et al. 2011). Disease caused by Leishmania infantum has already been diagnosed in dogs (Koutinas et al. 2010) and humans (Diza et al. 2008), while the spread of Phlebotomus spp. all over Greece has been studied in the past (Ntais et al. 2013). The objective of this preliminary study was to determine the prevalence of antibodies against WNV, A. ovis and L. infantum in dairy cattle from Northern Greece.

**Materials and methods**

The study was conducted from July to September 2010. Samples were taken from slaughterhouses of 4 prefectures in Northern Greece (Thessaloniki, Pella, Chalkidiki, Kilkis). In 2 of these prefectures (Thessaloniki, Pella), 101 cases of WNV encephalitis had been diagnosed in humans during the same summer (2010), while fewer similar cases (Kachrimanidou et al. 2011) had also been diagnosed in the other 2 prefectures (Kilkis, Chalkidiki) (Muma et al. 2011). During the outbreak in the summer of 2010 in Central Macedonia in Northern Greece, a total of 197 patients with neuroinvasive disease was reported, of whom 33 (17%) died according to data of the Hellenic Centre for Disease Control and Prevention1 (Danis et al. 2011).

In total, 156 blood samples were drawn before slaughter from adult cattle aged between 2 and 4 years. Fifty-six samples were drawn from cattle in Kilkis Prefecture, 54 samples in Thessaloniki Prefecture, 34 in Pella Prefecture and 12 in Chalkidiki Prefecture. Samples were kept refrigerated and the serum was separated by centrifugation at 2,500 x g for 20 min, approximately 1 hour after sampling. Sera were transferred in microcentrifuge tubes and stored at -20°C until analysis.

All samples were tested for antibodies against WNV, L. infantum and A. ovis.

IgG antibodies against WNV were tested using an ELISA commercial kit (ID screen West Nile competition ELISA kit, ID.VET, Montpellier, France), according to the manufacturer’s instructions. ELISA-positive samples were confirmed by using the plaque reduction neutralization test (PRNT) (OIE 2013).

Due to possible cross-reactions with other flaviviruses, positive sera were also tested for tick borne encephalitis virus (TBEV) [IMMUNOZIM® FSME (TBE) IgG All Species, Progen Biotechnik, GmbH, Heidelberg, Germany].

The presence of IgG antibodies against A. ovis was performed using an ELISA commercial kit (VMRD, Washington, USA), modified to detect A. ovis as suggested by others (Scoles et al. 2008).

Cattle sera were tested serologically using anti bovine, anti-IgG antibodies, by Indirect Immunofluorescence Test (IFAT, Leishmania SPOT IF, Santa Cruz, Brazil). A series of two-fold serum dilutions starting from 1/40 were performed. Based on our experience the surveyed area is considered endemic for canine leishmaniosis, so a cut-off titre of ≥ 1/160 was regarded as positive.

Data were collected and entered in a data sheet and analyzed using IBM SPSS 20.0 software for Windows (IBM SPSS Corp., Armonk, NY, USA). Chi-square (χ²) test, odd ration (OR) and the 95% confidence interval (CI) of the ORs were calculated to compare prevalence of antibodies against WNV, A. ovis and L. infantum among the four study areas. The p-value < 0.05 was considered statistically significant.

**Results**

Thirty out of 156 (18.6%) samples tested positive for WNV. All sera positive for WNV were negative for TBEV. Pella prefecture had significantly (p < 0.01) more positive samples compared to Thessaloniki (OR = 9.6; 95% CI = 3.02-31.67), Chalkidiki (OR = 15.71, 95% CI = 1.70-117) and Kilkis (OR = 28.6, 95% CI = 7.27-136.62), whereas the prevalence was not significantly different among the others prefectures (Table I). Table I shows also the WNV human cases recorded during 2010-2011, while the human cases and the cattle seropositivity to WNV in the same span of time are depicted in Figure 1.

Fifty-five out of 156 (35.9 %) samples were tested positive for Anaplasma ovis. Chalkidiki prefecture had the highest prevalence, here the infection rate for A. ovis was significantly higher (p < 0.01) compared to the other 3 prefectures [(Thessaloniki OR = 2.6, 95% CI = 1.09-6.23); (Kilkis OR = 11, 95% CI = 1.30-82.91); (Pella OR = 33, 95% CI = 4.29-156.71)]. Also, Thessaloniki had

Table I. Seroprevalence of West Nile virus (WNV), Anaplasma ovis (A. ovis) and Leishmania infantum (L. infantum) in greek cattle according to prefectures. Data on WNV human cases recorded during 2011-2012 originated from Hellenic Center for Disease Control & Prevention.

<table>
<thead>
<tr>
<th>Prefecture</th>
<th>Samples tested</th>
<th>WNV positive (%)</th>
<th>A. ovis positive (%)</th>
<th>L. infantum positive</th>
<th>WNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thessaloniki</td>
<td>54</td>
<td>7 (13)</td>
<td>15 (27.8)</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>Pella</td>
<td>34</td>
<td>20 (58.8)</td>
<td>1 (2.9)</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>Chalkidiki</td>
<td>12</td>
<td>1 (8.33)</td>
<td>11 (91.67)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Kilkis</td>
<td>56</td>
<td>2 (3.57)</td>
<td>28 (50)</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>30 (18.6)</td>
<td>55 (35.9)</td>
<td>0</td>
<td>126</td>
</tr>
</tbody>
</table>

Discussion

Lethal WNV encephalitis cases were reported in several mammalian species, including ruminants. In Northern Greece, human cases of the disease have been reported in the past (Kecskeméti et al. 2007, Papa et al. 2011a). Although the number of the samples tested was low, it is interesting that the seroprevalence for WNV was 18.6%, a rate much higher than the one reported in a similar study in Turkey 6 years ago, in which a 4% seroprevalence was detected (Ozkul et al. 2006). Most of the positive samples (27 out of 30, 90%) of this study were recorded in the prefectures of Thessaloniki and Pella, the 2 prefectures in which, most human cases of the disease were also detected. Therefore, cattle could be regarded as natural markers (positive or negative controls-sentinel animals) for this virus in high risk areas, as it already occurs for Bleutongue virus (Hoffmann et al. 2008).

The high prevalence of the virus in these areas could be the result of intense agricultural production and especially of rice, a product that needs a lot of water, an excellent background for increased numbers of wild birds and mosquitoes that transmit WNV (Papa et al. 2011a).

Anaplasma ovis was detected in 35.9% of the tested samples. Although in cattle the most pathogenic Anaplasma species are A. marginale and A. phagocytophilum (Durrani and Goyal 2012), A. ovis is the most commonly found Anaplasma in other ruminant species in Greece, it is potentially zoonotic and could cause disease in cattle (Chochlakis et al. 2010, Giadinis et al. 2011, Hornok et al. 2007, Hornok et al. 2012).

In the present study, all the examined samples were found negative for L. infantum. Low leishmaniosis seropositivity in cattle has been found in other
studies in Nepal (Bhattarai et al. 2010) and Bangladesh (Alam et al. 2011), countries that have high prevalence of human leishmaniosis.

In conclusion, cattle from Northern Greece seem to be negative to *L. infantum*, but they have a remarkable positivity for WNV and *A. ovis*. With regards to the latter, this means that they could serve as a possible hazard for the public health, as well as for the livestock of the infected areas. Apart from the results of the current study, the presence of vectors capable of transmitting and playing a role in the maintenance and circulation of these 2 pathogens in Northern Greece (Kachrimanidou et al. 2011, Ntais et al. 2013), should act as a hint for the application of public health surveillance schemes. As regards WNV, it could be suggested that cattle may serve as sentinels in areas at risk for this pathogen. In any way, molecular studies would be very important to confirm the results of the current survey and to intensify the prophylactic measures (e.g. application of proper and continuous disinfection procedures) in the herds.

References


