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

Nektarios D. Giadinis[1*], Panagiotis D. Katsoulos[2], Dimosthenis Chochlakis[2,3], Yannis Tselentis[2,3], Pantelis Ntais[2], Shawkat Q. Lafi[4], Harilaos Karatzias[1] & Anna Psaroulaki[2]

[1] *Clinic of Farm Animals, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, St. Voutyra 11, 546 27 Thessaloniki, Greece.*
[2] *Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, University of Crete, Voutes-Staurakia, 71110 Heraklion, Greece.*
[3] *Regional Laboratory of Public Health of Crete, Voutes-Staurakia, 71110 Heraklion, Greece.*
[4] *Department of Pathology and Public Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan.*

** Corresponding author at: Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University, St. Voutyra 11, 54627 Thessaloniki, Greece.*
Tel.: +30 2310 994509, e-mail:ngiadini@vet.auth.gr.

Veterinaria Italiana 2015, **51** (3), 205-209. doi: 10.12834/VetIt.174.523.4
Accepted: 19.04.2014 | Available on line: 30.09.2015

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

Summary
This study investigates the seroprevalence of Greek adult cattle against West Nile virus, *Anaplasma ovis* and *Lehismania 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.

Sieroprevalenza delle infezioni da West Nile virus, Anaplasma ovis *e* Leishmania infantum *in bovini adulti nel Nord della Grecia*

Parole chiave
Anaplasma ovis,
Bovino,
Grecia,
Lehismania 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 *Lehismania 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, Salonicco e Pella, erano stati riportati, precedentemente a questa indagine, casi di West Nile nell'uomo. Trenta (18,6%) dei campioni analizzati sono risultati positivi al virus della West Nile e 55 (35,9%) ad *Anaplasma ovis*. Tutti i 156 campioni sono risultati negativi a *Leishmania infantum*. Le prefetture in cui sono stati prelevati i campioni positivi al virus West Nile sono risultate le stesse in cui il virus è stato isolato nell'uomo. Questo riscontro impone di considerare i bovini come indicatori dell'attività del virus in zone ad alto rischio.

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.

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 Prevention[1] (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*.

[1] http://www.keelpno.gr/en-us/home.aspx.

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 (χ^2) 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.

Prefecture	Animal population				Human cases
	Samples tested	WNV positive (%)	*A. ovis* positive (%)	*L. infantum* positive	WNV
Thessaloniki	54	7 (13)	15 (27.8)	0	63
Pella	34	20 (58.8)	1 (2.9)	0	46
Chalkidiki	12	1 (8.33)	11 (91.67)	0	5
Kilkis	56	2 (3.57)	28 (50)	0	12
Total	156	30 (18.6)	55 (35.9)	0	126

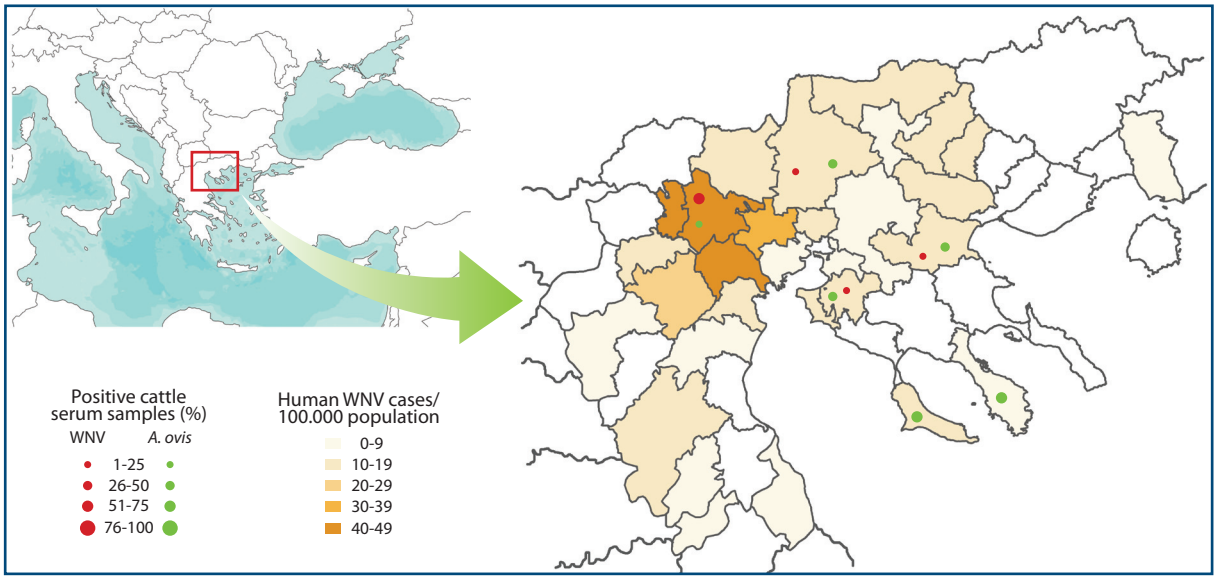

Figure 1. *Human West Nile virus (WNV) cases and cattle WNV and* Anaplasma ovis *(A.ovis) seroprevalence in Northern Greece in 2010-2011.*

significantly higher prevalence ($p < 0.05$) compared to Pella (OR = 12.69, 95% CI = 1.59-82.67). Kilkis had significantly higher prevalence rate ($p < 0.05$) compared to Thessaloniki (OR = 2.6, 95% CI = 1.09-6.23) and to Pella (OR = 12.69, 95% CI = 1.59-82.67) $p< 0.01$ (Table I).

Samples tested for *L. infantum* yielded negative results.

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 disinfestation procedures) in the herds.

References

Aktas M., Altay K. & Dumanli N. 2011. Molecular detection and identification of *Anaplasma* and *Ehrlichia* species in cattle from Turkey. *Ticks Tick borne Dis*, **2**, 62-65.

Alam M.S., Ghosh D., Khan Md.G.M., Islam M.F., Mondal D., Itoh M., Islam Md.N. & Haque R. 2011. Survey of domestic cattle for anti-*Leishmania* DNA in a visceral leishmaniosis endemic area of Bagladesh. *BMC Vet Res*, **7**, 27.

Bhattarai N.R., Van der Auwera G., Rijal S., Picado A., Speybroeck N., Khanal B., de Doncker S., Lai Das M., Ostyn B., Davies C., Coosemans M., Berkvens D., Boelaert M. & Dujardin,J.-C. 2010. Domestic animals and epidemiology of visceral leishmaniosis, Nepal. *Emerg Infect Dis*, **16**, 231-237.

Chaskopoulou A., Dovas C.I., Chaintoutis S.C., Bouzalas I., Ara G. & Papanastassopoulou M. 2011. Evidence of zoonotic circulation of West Nile virus (Nea Santa-Greece-2010, lineage 2), Greece, May to July 2011. *Eurosurveill*, **16**, pii=19933.

Chochlakis D., Ioannou I., Tselentis Y. & Psaroulaki A. 2010. Human anaplasmosis and *Anaplasma ovis* variant. *Emerg Infect Dis*, **16**, 1031-1032.

Danis K., Papa A., Theocharopoulos G., Dougas G., Athanasiou M., Detsis M., Baka A., Lytras T., Mellou K., Bonovas S. & Panagiotopoulos T. 2011. Outbreak of West Nile virus infection in Greece, 2010. *Emerg Infect Dis*, **17**, 1868-1872.

de la Fuente J., Ruiz-Fons F., Naranjo V., Torina A., Rodríguez O. & Gortázar C. 2008. Evidence of *Anaplasma* infections in European roe deer (*Capreolus capreolus*) from southern Spain. *Res Vet Sci*, **84**, 382-386.

Diza E., Kansouzidou A., Gerou S., Vezyri E., Metallidis S. & Antoniadis A. 2008. Leishmaniases in Northern Greece: seroprevalence of the infection and incidence of the disease during the period 2001-2006. *Eur J Clin Microbiol Infect Dis*, **27**, 997-1003.

Durrani A.Z. & Goyal S.M. 2012. A retrospective study of *Anaplasma* in Minnesota cattle. *Turk J Vet Anim Sci*, **36**, 131-136.

Giadinis N., Chochlakis D., Tselentis Y., Petridou E., Karatzias H. & Psaroulaki, A. 2011. Acute and chronic anaplasmosis due to *Anaplasma ovis* infection in dairy goats. *In* Proceedings of the 6th International Meeting on *Rickettsiae* and Rickettsial Diseases, Crete, Greece.

Hoffmann B., Sasserath M., Thalheim S., Bunzenthal C., Strebelow G. & Beer M. 2008. Bluetongue virus serotype 8 reemergence in Germany, 2007 and 2008. *Emerg Infect Dis*, **14**, 1421-1423.

Hornok S., Elek V., De la Fuente J., Naranjo V., Farkas R., Majoros G. & Földvári G. 2007. First serological and molecular evidence on the endemicity of *Anaplasma ovis* and *A. marginale* in Hungary. *Vet Microbiol*, **122**, 316-322.

Hornok S., Micsutka A., Fernández de Mera I.G., Melil M.L., Gönczi E., Tánczos B., Mangold A.J., Farkas R., Lutz H., Hofmann-Lehmann R. & De la Fuente J. 2012. Fatal bovine anaplasmosis in a herd with new genotypes of *Anaplasma marginale, Anaplasma ovis* and concurrent haemoplasmosis. *Res Vet Sci*, **92** (1), 30-35.

Kachrimanidou M., Papa A., Chochlakis D., Pavlidou V. & Psaroulaki A. 2011. Molecular evidence for *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks from Greece. *Vector Borne Zoonotic Dis*, *11*, 1391-1393.

Kecskeméti S., Bajmócy E., Bacsadi A., Kiss I. & Bakonyi T. 2007. Encephalitis due to West Nile virus in a sheep. *Vet Rec*, **161**, 568-569.

Koutinas A.F., Carlotti D.N., Koutinas C., Papadogiannakis E.I., Spanakos G.K. & Saridomichelakis M.N. 2010. Claw histopathology and parasitic load in natural cases of canine leishmaniosis associated with *Leishmania infantum*. *Vet Dermatol*, **21**, 572-577.

Muma J.B., Munyeme M., Matope G., Siamudaala V.M., Munang'andu H.M., Matandiko W., Godfroid J., Skjerve E. & Tryland M. 2011. *Brucella* seroprevalence of the Kafue lechwe (*Kobus leche kafuensis*) and Black lechwe (*Kobus leche smithemani*): exposure associated to contact with cattle. *Prev Vet Med*, **100**, 256-260.

Ntais P., Sifaki-Pistola D., Christodoulou V., Messaritakis I., Pratlong F., Poupalos G. & Antoniou M., 2013. Leishmaniases in Greece. *Am J Trop Med Hyg*, **89**, 906-915.

World Organisation for Animal Health (OIE). 2013. West Nile fever, Chapter 2.1.20 Manual of diagnostic tests and vaccines for terrestrial animals, OIE, Paris, France, 6.

Ozkul A., Yildirim Y., Pinar D., Akcali A., Yilmaz V. & Colak,

D. 2006. Serological evidence of West Nile virus (WNV) in mammalian species in Turkey. *Epidemiol Infect*, **134**, 826-829.

Papa A., Xanthopoulou K., Gewehr S. & Mourelatos S. 2011a. Detection of West Nile Virus lineage 2 in mosquitos during a human outbreak in Greece. *Clin Microbiol Infect*, **17**, 1176-1180.

Papa A., Bakonyi T., Xanthopoulou K., Vásquez A., Tenorio A. & Nowotny N. 2011b. Genetic characterization of west Nile Virus lineage 2, Greece, 2010. *Emerg Infect Dis*, **17**, 920-922.

Scoles G.A., Goff W.L., Lysyk T.J., Lewis G.S. & Knowles D.P. 2008. Validation of an *Anaplasma marginale* cELISA for use in the diagnosis of *A. ovis* infections in domestic sheep and *Anaplasma* spp. in wild ungulates. *Vet Microbiol*, **130**, 184-190.

Singh N., Mishra J., Singh R. & Singh S. 2013. Animal reservoirs of visceral leishmaniosis in India. *J Parasitol*, **99**, 64-67.

Valiakos G., Touloudi A., Iacovakis C., Athanasiou L., Birtsas P., Spyrou V. & Billinis C. 2011. Molecular detection and phylogenetic analysis of west Nile virus lineage 2 in sedentary wild birds (Eurasian magpie), Greece, 2010. *EuroSurveill*, **16**, pii: 19862.