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Short communication

Factors associated with IgG positivity to Crimean-Congo hemorrhagic fever virus in the area with the highest seroprevalence in Greece

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ABSTRACT

In order to gain insight into the factors playing a role for the high seroprevalence of Crimean-Congo hemorrhagic fever virus in the human population of Thesprotia prefecture, Greece, serum samples were collected from residents of the area together with a questionnaire about demographic and epidemiological factors. A 14.4% seroprevalence was detected, with increased age, agro-pastoral activities, slaughtering, and contact with animals (especially sheep) among the factors associated with seropositivity. The high seroprevalence with the absence of any clinical cases needs further investigation.

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a viral tick-borne disease endemic in several regions of Asia, Africa, and Europe. It is of great public health concern as (i) it is a severe human disease with a fatality rate of up to 30%, (ii) the causative virus (CCHFV) is transmitted to humans by several modes, through the bite of infected ticks (mainly *Hyalomma* spp.) or direct contact with blood or tissues of viremic patients or animals, and (iii) there is a high risk of nosocomial outbreaks (Papa, 2010). A number of risk factors for acquiring the infection play a role, which vary from country to country (e.g. tick bite in Turkey, slaughtering and animal contact in Iran) (Chinikar, 2007; Vatansever et al., 2007). The CCHF epidemiology is complicated, since although *Hyalomma* spp. ticks are widely distributed, the disease occurs only in specific foci, while sometimes the disease emerges in countries with no previous history of CCHF, with thousands of cases after this emergence (like in Turkey). This is a result of a complex enzootic cycle of CCHFV, which is influenced by a combination of interactions resulting in a focal geographic distribution range which does not always coincides with that of the vector species (Estrada-Peña et al., 2012).

Greece is a country in the Balkan peninsula, bordering with known CCHF endemic countries, like Bulgaria and Albania, where sporadic cases and outbreaks have been reported soon after the first description of the disease in Crimea up to nowadays (Papa et al., 2002, 2004). However, CCHF cases have not been reported from Greece, apart one (fatal) case observed in 2008 (Papa et al., 2010). Results of a recent study showed an overall seroprevalence of 4.2%, with significant differences between prefectures (range 0–27.5%); the highest rate (11/40, 27.5%) was detected in Thesprotia prefecture, a mountainous area in northwestern Greece (Sidira et al., 2012). Since only 40 samples had been tested from that area, the aim of the present study was to check in more detail the CCHFV situation in this area and find out any risk factors associated with seropositivity.

Materials and methods

Serum samples were collected during 2010–2012 from 166 persons (71 males, 42.8%) permanent residents of Thesprotia prefecture, northwestern Greece (Fig. 1). The age of the participants ranged from 18 to 90 years (median 66 years), and they were randomly selected among persons who visited the local health centers. All were informed about the study and were interviewed using a questionnaire about place of residence, age, sex, occupation,

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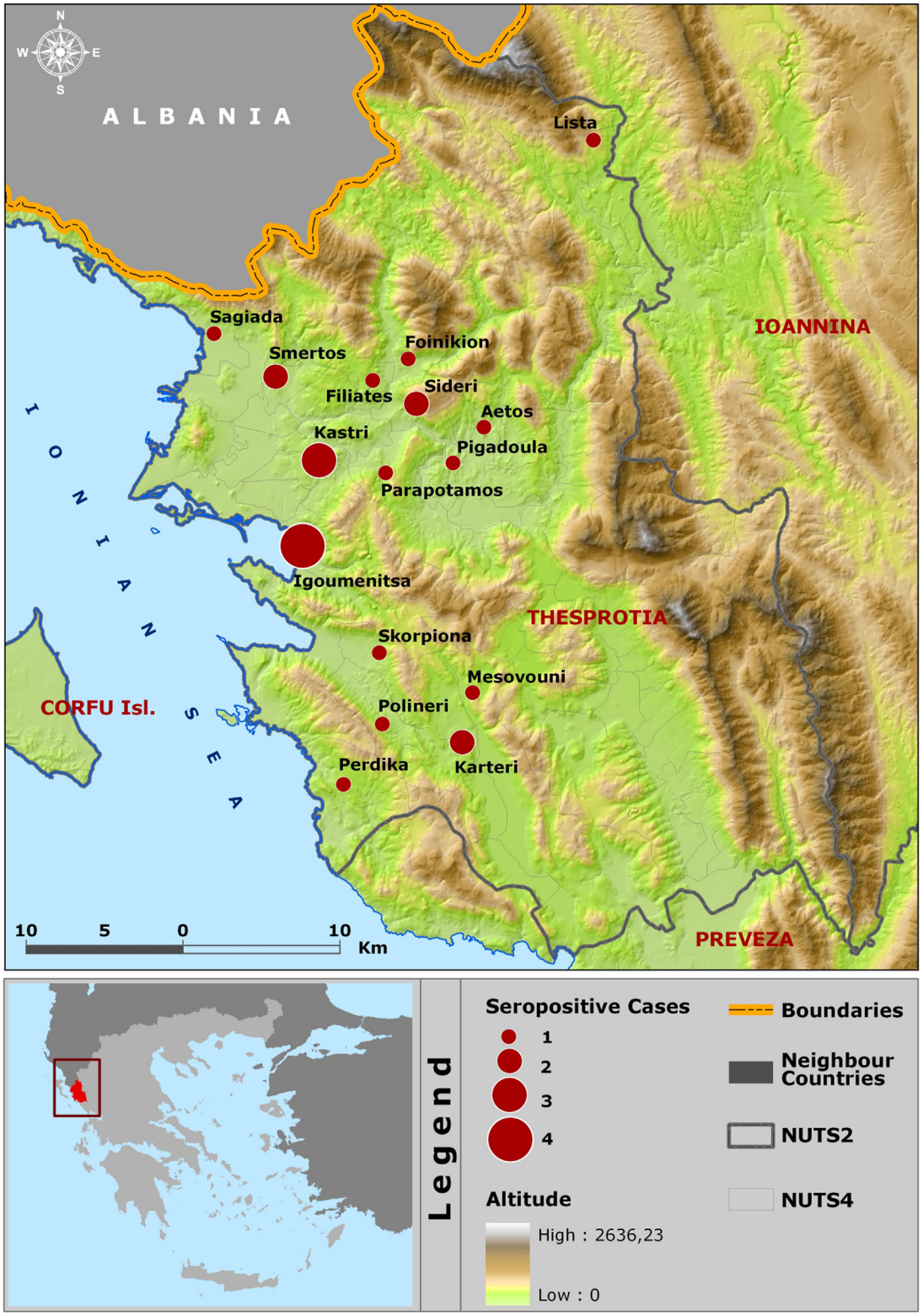

Fig. 1. Sites of residence of persons who were IgG-positive for Crimean-Congo hemorrhagic fever virus in Thesprotia prefecture in northwestern Greece (inset), 2010–2012. The size of the circle indicates the number of persons with positive test results in each location.

contact with animals, history of tick bite, and other activities or factors related with increased risk of CCHF infection. They all provided written consent before participating. The study was approved by the Ethics Committee of the Medical School of Aristotle University of Thessaloniki.

Serum samples were tested for CCHFV IgG antibodies by ELISA (Vektor-Best, Koltsovo, Novosibirsk, Russia) following the instructions of the manufacturer. All the IgG-positive samples were tested also for CCHFV IgM antibodies using ELISA from the same company. The IgG-positive samples were further tested

Table 1
Univariate logistic regression analysis of CCHFV seropositivity in association with risk factors in Thesprotia prefecture, northwestern Greece.

	CCHFV IgG		*p*	OR	95% CI
	Positive (*n* = 24)	Negative (*n* = 142)			
Age, Median (min, max)	75.67 (37, 88)	59.82 (18, 90)	<0.001	1.07	1.03, 1.12
Sex			0.906	0.95	0.40, 2.28
Male	10 (14.1%)	61 (85.9%)			
Female	14 (14.7%)	81 (85.3%)		Reference	
Occupation			**0.026**	4.17	1.19, 14.65
Agro-pastoral	21 (19.1%)	89 (80.9%)			
Other	3 (5.4%)	53 (94.6%)		Reference	
Contact with goats/sheep/cattle			**0.035**	3.35	1.09, 10.33
Yes	20 (19.0%)	85 (81.0%)			
No	4 (6.6%)	57 (93.4%)		Reference	
Contact with goats			0.456	1.40	0.58, 3.38
Yes	10 (17.2%)	48 (82.8%)			
No	14 (13%)	94 (87%)		Reference	
Contact with sheep			**0.012**	3.32	1.30, 8.51
Yes	17 (22.1%)	60 (77.9%)			
No	7 (7.9%)	82 (92.1%)		Reference	
Contact with cattle			0.906	0.93	0.25, 3.40
Yes	3 (13.6%)	19 (86.4%)			
No	21 (14.6%)	123 (85.4%)		Reference	
Altitude, mean (IQR)	159 (5–460)	142 (5–511)	0.502	1.00	1.00, 1.01
Slaughtering			**0.030**	2.69	1.10, 6.55
Yes	11 (24.4%)	34 (75.6%)			
No	13 (10.7%)	108 (89.3%)		Reference	
History of tick bite			0.855	1.09	0.45, 2.60
Yes	14 (14.9%)	80 (85.1%)			
No	10 (13.9%)	62 (86.1%)		Reference	

Significant *p* values are shown in bold.

using the kit Crimean-Congo Fever Mosaic 2 *IFA* (Euroimmun Medizinishe Labordiagnostika AG, *Lübeck*, Germany) which is based on the biochip technology using recombinant glycoprotein precursor (GPC) and nucleoprotein (N) antigens.

Statistical analysis was performed using the IBM SPSS 19.0 statistical package. The relationship between the categorical variables was assessed using chi-square test or Fisher's exact test (when the expected count of cells was <5). The potential association of several predictors to CCHFV seropositivity was investigated via generalized linear modeling, mostly through multiple logistic regression. The statistical robustness was checked using the probit and cloglog links. Model adequacy was assessed via the Akaike information criterion and by visually inspecting the deviance residuals. Odds ratio (OR) and 95% confidence intervals (95% CIs) were obtained to identify factors associated with CCHFV seropositivity. Statistical significance was defined as a two-tailed *p* value of less than 0.05.

Results

Among the 166 persons tested, 24 (14.4%) were found to carry CCHFV IgG antibodies. Seroprevalence did not differ between males and females ($p > 0.05$); however, age was an important factor, with increased age related significantly with seropositivity ($p < 0.001$). The age factor remained significant even in the multivariate analysis. Univariate analysis showed that apart from increased age, agro-pastoral activities, slaughtering, and contact with animals (mainly sheep) were associated with CCHFV seropositivity (Table 1). In general, the model appeared to be robust in the sense that alternative link functions like probit and cloglog provided essentially identical results. Therefore, the results of the logistic model are presented, which corresponds to the canonical link function.

All IgG-positive persons were IgM-negative, except one, in whom low titers of IgM antibodies were detected by ELISA and IFA. That person did not recall any symptoms resembling CCHF.

All the ELISA IgG-positive samples were positive also by the IFA, either to GPC or N antigen, or to both of them. Specifically, 7 were positive only to N antigen, 6 only to GPC, and 11 in both GPC and N antigens.

Discussion

Thesprotia is the most northwestern, and one of the most sparsely populated prefectures of Greece, with 43,587 inhabitants (according to the 2011 census), most people (71.2%) living in rural areas. The total area of 1515 km^2 is mainly mountainous (43.7%) and semi-mountainous (48%), and only 8.3% are lowland. During a recent screening for CCHFV IgG seroprevalence in Greece, unexpectedly high rates were observed in the mountainous areas (Sidira et al., 2012). In the present study, 166 persons from Thesprotia prefecture were interviewed and tested for CCHFV IgG antibodies. A seroprevalence of 14.4% was detected, which, although lower than that in the initial study, it is among the highest reported. Even among a high-risk population in the most endemic provinces in Turkey (Tokat and Sivas), a 12.8% seroprevalence was reported (Gunes et al., 2009). It is difficult to explain the high seroprevalence, since CCHF cases had never been reported in this area, while none of the participants recalled any symptom resembling CCHF. None of the persons with a history of tick bite reported any kind of bleeding or high fever, and the only symptom reported by few of them was local redness at the site of the bite. A recent study in Turkey showed that 88% of CCHFV infections are subclinical (Bodur et al., 2012). Probably the infections in Greece are subclinical, related with a low or non-pathogenic CCHFV strain. Usually the CCHFV IgG antibodies persist for a long time, explaining the fact that increased age is significantly associated with the seropositivity.

Livestock breeding is well developed in Thesprotia, with sheep, goat, and cattle population densities of 113.74, 57.63, and 18.21 per km^2, respectively (the human population density is 28.77 per km^2) (Hellenic Statistical Authority – ELSTAT, 2011). A significant relation between seropositivity and contact with sheep/goats/cattle was observed ($p = 0.035$), and especially with

sheep ($p = 0.012$). Serological studies on one-year-old sheep will show the situation in animals and elucidate their role in CCHF epidemiology in the region and in general.

Approximately one third of the area (457 of the total 1515 km^2) is covered by a combination of shrub and herbaceous vegetation, with only 4.7 km^2 covered by residential buildings. Furthermore, there is a habitat fragmentation, and previous studies showed that this is the key factor driving the spread of CCHF (Estrada-Peña et al., 2010).

The residence sites of most (20/24) seropositive persons were in rural areas at the foothills of the mountains, while 4 were residents of Igoumenitsa, the capital and port city of the prefecture (Fig. 1). Although these 4 were residents of an urban area, all reported a previous tick bite, 3 were stock breeders with sheep grazing in the nearby hillsides, and one was a slaughterer. The majority of the population in Thesprotia has agro-pastoral activities, even if not as the main occupation, and it was found that they were at 4.17 times higher risk of acquiring CCHFV infection, while slaughterers were at 2.69 times higher risk. However, the high seropositivity with the absence of CCHF case reports suggests that either the cases were undiagnosed or the infections were caused by a strain of low pathogenicity. In the latter case, the factors playing a role for the CCHFV seropositivity in Thesprotia prefecture could be considered as protection rather than risk factors. Well-designed studies are needed on ticks and febrile cases to elucidate this unique situation in Greece.

Conflicts of interest

There are no conflicts of interest.

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