

Seroprevalence survey of arboviruses in workers from Tuscany, Italy

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PAROLE CHIAVE: Virus Toscana; virus West Nile; virus dell'encefalite da zecca; virus Usutu; infezioni occupazionali; sieroprevalenza

SUMMARY

Background: *Arthropod-borne viruses (Arbovirus) play an important role among emerging and re-emerging infectious diseases and in the spreading of infections in new geographic areas. Although some arboviral infections may be asymptomatic or mild flu-like illnesses, many occur as severe forms of meningitis and meningoencephalitis. Objectives:* To assess whether arboviral infections may be associated with occupational risk, in a population of agricultural and forestry workers potentially at high risk for arthropods bite and sting. **Methods:** A seroprevalence survey for arboviruses belonging to the genera *Flaviviruses* (West Nile, Tick-borne encephalitis and Usutu viruses) and *Phlebovirus* (Toscana virus) was carried out in Grosseto province (Tuscany, Italy). One hundred and one serum samples of occupationally exposed workers and 100 serum samples of not exposed workers were analyzed using commercial and home-made serological assays. Serological data were obtained in 2012 and analyzed according to demographic characteristics, recollection of insect-bites, and time spent in outdoor activities. **Results:** A total seropositivity of 10% (21/201) was observed for Toscana virus. No difference in seroprevalence for Toscana virus was observed among the exposed (10/101) versus the not exposed (11/100) workers. No seropositivity for West Nile, Usutu and Tick-borne encephalitis viruses was detected. **Conclusions:** Although circulation of Toscana virus is recognized in the study area, our results did not reveal a higher risk for workers exposed to arthropods bite and sting. Health surveillance programs remain useful to monitor the potential emergence of arboviruses.

RIASSUNTO

«**Studio di sieroprevalenza di Arbovirus in lavoratori della Toscana, Italia**». **Introduzione:** I virus trasmessi da artropodi (*Arbovirus*) hanno un ruolo importante nelle malattie infettive emergenti e riemergenti e nella diffusione di infezioni virali in nuove aree geografiche. Sebbene alcune di queste infezioni possano presentarsi in modo asintomatico o simil-influenzale, molte di esse possono causare gravi forme di meningiti o meningoencefaliti. **Obiettivi:** Al fine di valutare se queste infezioni virali possano essere associate ad un rischio occupazionale è stato condotto uno

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studio di sieroprevalenza per *Arbovirus* appartenenti ai generi *Flavivirus* (*West Nile*, *Tick-borne encephalitis* e *Usutu virus*) e *Phlebovirus* (*Toscana virus*) in una popolazione professionalmente esposta, ad alto rischio per puntura e morso di artropodi (agricoltori e forestali). Questi studi sono stati condotti nella provincia di Grosseto, Toscana, dove si sono verificate epidemie di questi virus. **Metodi:** Nel 2012 sono stati raccolti e analizzati 101 campioni di lavoratori professionalmente esposti al morso e alla puntura di artropodi per attività lavorativa e 100 campioni di siero di lavoratori non esposti professionalmente, mediante saggi sierologici sia home-made che commerciali. I dati anamnestici raccolti da tutti i soggetti arruolati nello studio riguardavano, oltre alle caratteristiche demografiche, il ricordo di punture di insetti e la durata delle attività all'aperto. **Risultati:** È stata osservata una sieroprevalenza del 10% per il *Toscana virus*. Tuttavia non sono state osservate differenze tra il gruppo di lavoratori esposti (10 positivi su 101 soggetti) e il gruppo dei non esposti professionalmente (11 positivi su 100 soggetti). Non è stata inoltre riscontrata sieropositività per i virus *West Nile*, *Usutu* e *Tick-borne encephalitis*. **Conclusioni:** Sebbene la circolazione del virus *Toscana* sia ben documentata nell'area di studio, i nostri risultati non hanno evidenziato un rischio maggiore per i lavoratori esposti alla puntura e al morso di artropodi. Studi di sorveglianza sono da ritenersi rilevanti per la sanità pubblica e occupazionale per fornire informazioni precoci sulla potenziale emergenza di *Arbovirus* in aree geografiche specifiche.

INTRODUCTION

Arthropod-borne viruses (Arboviruses) are the causative agents of significant morbidity and mortality among humans and domestic animals worldwide, playing an important role in the emergence and re-emergence of infectious diseases and in the spread of viral infections in new geographic areas. Although some arboviral infections may cause asymptomatic or mild flu-like illness, many occur as severe forms of meningitis and meningoencephalitis.

The geographical position of Italy, with heterogeneous climatic zones, favors the wide distribution of different species of arthropods that act as possible vectors of human pathogens. Climatic changes and the rise of the environmental temperature in recent years have increased the density of vector populations, resulting in the spreading of viruses transmitted by them (22). The migration of some species of birds and the interaction between viruses and the potential reservoirs could play a significant role in the emergence of some arboviral infections. Among Arboviruses, of particular importance are those belonging to the genus *Flavivirus*, family *Flaviviridae* such as *West Nile* (WN), *Tick-Borne Encephalitis* (TBE) and *Usutu* (USU) viruses, and to the genus *Phlebovirus*, family *Bunyaviridae* such as *Toscana*

(TOS) virus that are responsible of meningitis and meningoencephalitis (14, 27, 33).

WN virus was first detected in 1937 in the blood of a woman in the West Nile Province of Uganda (30). WN virus has a broad geographic distribution (12), having been described in Africa, Europe, Middle East, Asia, Oceania, Australia and North America. The WN virus is maintained in natural transmission cycles involving birds and mosquitoes, primarily the *Culex* species. Human infection is usually mild or asymptomatic; severe disease is commonly reported in older patients (12). In Italy, entomological, veterinary, and human surveillance systems for WN virus infection have been implemented starting from 1998, when the disease was first detected in horses in Tuscany. No further outbreaks were described until the late summer 2008, when human cases were reported in north-eastern Italy, now considered an endemic area for this virus. In the 2008–2012 period, WN virus foci were also reported in central-southern regions of Italy and in the Islands, with 73 human cases with confirmed neuro-invasive disease (6, 9, 24, 34). More recently, from June to October 2016, 71 confirmed cases were reported (17). Entomological and epidemiological surveillance has highlighted the circulation of both lineage 1 and 2 starting from 2011, when lineage 2 strain determined human cases (6, 28, 29).

In Italy, WN and USU viruses were simultaneously found in sentinel animals and mosquitoes (23, 28). USU virus was discovered in South Africa in 1959. In Europe, the first demonstration of USU virus circulation was reported in 2001 in Austria, with a significant mortality of Eurasian blackbirds. In subsequent years, the virus has spread to neighboring countries, including Italy, Germany, Spain, Hungary, Switzerland, Poland, England, Czech Republic, Greece and Belgium, causing an unusual mortality in birds. In 2009, the first 2 cases of USU virus invasive infection in humans were described in Italy (10, 18, 25, 32).

TBE virus is transmitted to humans by tick-bite, causing an infection characterized by a biphasic febrile illness, often with neurological manifestations of meningoencephalitis. Every year it is responsible for cases of meningitis and meningoencephalitis in northern Italy (7). First isolated in 1937 in the former Soviet Union, in Europe TBE has been known since 1931, when an outbreak of acute epidemic serious meningitis was reported in south-eastern Austria. Currently, this infection is endemic in 27 European countries. Worldwide, the incidence of clinical cases is estimated between 10,000 and 15,000 per year, though it appears to be increasing (16). At the beginning of the 1980s, a TBE focus was described for the first time in Italy, in the Florence province, with sporadic cases occurring in the subsequent years (34). From the 1990s, TBE is endemic in some north-eastern provinces (Trento, Bolzano, Belluno, Pordenone, Udine, Treviso and Vicenza), where the mean annual incidence increased from 0.06/100,000 in 1992 to 0.88/100,000 in 2006 (3). In the 1990s in the Belluno area, after the confirmation of autochthonous cases of infection, vaccination against TBE was implemented. Two TBE vaccines are available in Europe: FSME-Immun R[®] (Baxter Innovations GmbH, Vienna, Austria) and Encepur R[®] (Novartis Vaccines and Diagnostics GmbH & Co., KG, Marburg, Germany) (19, 21). The protective immunity rate of the vaccines is very high (96-98%), according to field studies in Austria (20). Currently, the conventional vaccine schedule recommends a primary series of three doses and a booster every 3-5 years.

TOS virus (TOSv) was originally isolated from the sandfly *Phlebotomus perniciosus*, collected in cen-

tral Italy in 1971. Following the discovery, TOSv was shown to be endemic in several other regions, where the insect vectors (*P. perniciosus* and *Phlebotomus perfiliewi*) are present (11). Other Mediterranean countries, including Spain, France, Portugal, Cyprus, Greece and Turkey, are considered among the endemic regions (1). TOSv represents a widespread public health problem, since it may be associated with acute neurological diseases, mainly aseptic meningitis. Under favorable environmental and climatic conditions, this virus can infect humans, depending on the life cycle of the phlebotomine sandflies vectors (15). Asymptomatic infections and infections without central nervous system involvement have also been described (8).

Since Arboviruses are transmitted to humans primarily through the bite of insect vectors, in recent years the occurrence of these infections have been extensively studied in Italian populations considered at risk because of their activities in outdoor environment (15).

The document of the Italian Ministry of Health (Surveillance for human cases of Chikungunya, Dengue, West Nile disease and other arbovirolosis and risk assessment of transmission in Italy-2015-2017) (13) describes the current spread of these arboviruses and the control measures to be implemented. We attempted to assess whether these viral infections may be associated with occupational exposures through a serosurvey in workers from Grosseto province, Tuscany, where the circulation of arboviruses belonging to the genera *Flavivirus* and *Phlebovirus* has been documented in the past years (4, 15, 31).

METHODS

From January to May 2012, during periodical health surveillance surveys offered to workers by the local health authority, aimed at active search for cases of occupational disease, 201 blood samples were taken from healthy subjects residing in the Grosseto area, including 101 from workers involved in agricultural and forestry activities potentially exposing to arthropods bites and stings, and 100 from occupationally active not exposed subjects employed in public health offices, living in the same areas. The health surveillance practice is a voluntary campaign

and occupational physicians asked workers written informed consent to participate in this investigation before blood drawing.

Socio-demographic and personal data, information on type of job and lifestyle associated with environmental exposure other than working environment (home places with garden, frequent outdoor activities, etc.) were collected through structured interviews, using a validated questionnaire. All participants were recruited on the occasion of the periodical health surveillance program and they gave a written informed consent.

Laboratory procedures

Sera were collected from all subjects and stored at -20°C until laboratory analysis. All samples were serologically tested for WN, USU, TBE and TOS viruses infections. WN and TBE antibodies were analyzed by commercial IgG Enzyme-Linked Immunosorbent Assays (ELISAs) (DxSelect Focus Diagnostics, California, USA and FSME IgG Immunozym Progen Biotech GMBH, Heidelberg, Germany, respectively). A home-made ELISA was used for TOSv as previously described (8). In order to evaluate the specificity of the antibody response, all ELISA positive sera were confirmed by neutral-

izing antibodies analysis using Plaque Reduction Neutralization Test (PRNT) as previously described (2, 26). Briefly: inactivated serum samples were diluted and mixed with an equal volume containing 80 PFU of virus/100 μl , incubated overnight at 4°C and inoculated into cells monolayers (PK15 cells for USU and TBE viruses and VERO cells for TOS and WN viruses). An $>80\%$ reduction in number of plaques was selected as criterion for virus neutralizing titers.

Statistical analysis

We calculated odds ratios (OR) and 95% confidence intervals (CI) of seroprevalence according to selected individual characteristics using unconditional univariable and multivariable logistic regression. Statistical analysis was performed using the software EpiInfo.

RESULTS

Exposed and not exposed workers were categorized by age, sex, vaccination history, demographic and working characteristics, as shown in table 1. Most participants came from the city of Grosseto

Table 1. Characteristics of the exposed (E, n. 100) and not exposed (NE, n. 101) workers

Variables	Sub-category	E n (%)	NE n (%)	p-value
Age	<40 ys	50 (49.5)	8 (8.0)	0.0000
	41-50 ys	22 (21.8)	38 (38.0)	0.0001
	>50 ys	29 (28.7)	54 (54.0)	0.003
Sex	Male	80 (79.2)	52 (52.0)	0.00007
	Female	21 (20.8)	48 (48.0)	
Type of job	Farmer	96 (95.1)		
	Executive	4 (3.9)	2 (2.0)	
	Clerical worker		30 (30.0)	
	Health care worker		44 (44.0)	
	Other	1 (1.0)	24 (24.0)	
Country of origin	Italy	81 (80.2)	98 (98.0)	0.0001
	Other	20 (19.8)	2 (2.0)	
House with garden	Yes	37 (36.6)	43 (43.0)	0.36
	No	64 (63.4)	57 (57.0)	

or from the surroundings within a range of 50 km. Mean age of exposed workers was 42.30 years (range 21-67 years), and 79.2% were male. Mean age of not exposed workers was 50.26 (range 21-63 years), and 52.0% were male.

Albania and Romania were the most represented countries of origin of agricultural workers coming from abroad (20% of exposed workers versus 2% of not exposed subjects).

The survey gained also information on use of personal protective equipment (PPE) (gloves, safety shoes, face shields, protective clothes and protective hats) among agricultural workers. Results showed a good compliance with PPE, ranging from 65% (hats) to 97% (gloves).

Laboratory analysis showed one sample from exposed workers and three from not exposed workers IgG-reactive by ELISA for WN virus, but the positivity was not confirmed for WN and USU viruses by PRNT. Furthermore, the neutralization test did not confirm three positive and two borderline sera for TBE virus by ELISA test. Twenty-five samples were positive and 21 borderline for TOSv IgG antibodies, but only 21 specimens (10 from exposed workers and 11 from not exposed workers) resulted positive by PRNT. No significant difference (table

2) was observed between the two groups with regard to gender, age, house with garden and working exposure (OR 0.91, 95% CI, 0.368-2.247). There was no clinical evidence of recent infection of TOS virus in any of the detected cases. It should be noted that 7/10 exposed and 8/11 not exposed workers practiced extra-occupational outdoor activities.

DISCUSSION

An epidemiological picture of arboviral infections is missing in the occupational field, even if some of these viruses are known to circulate in Italy since decades. Assessing the occupational risk is important not only for protecting workers' health, but also for providing information on the potential spread of Arboviruses. It should be taken into account that many arboviral infections are subclinical or misdiagnosed, the acute neurologic illness being only "the tip of the iceberg". Few studies on the occupational risk caused by infected arthropods (*Flaviviruses* transmitted by mosquitoes and ticks, and *Phlebotomus* transmitted by sandflies) have been carried out (15, 31).

Starting from the evidences that TBE virus is now endemic in some regions of northern Italy

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) of TOSV seroprevalence according to selected characteristics

Variable	N positive	%	OR-1*	95%CI-1*	OR-2**	95%CI-2**
Gender						
Male	16	12.1	1.00	(Reference)	1.00	(Reference)
Female	5	7.2	0.566	0.198-1.618	0.499	0.165-1.507
Age (years)						
<40	5	8.6	1.00	(Reference)	1.00	(Reference)
40-49	11	13.3	0.964	0.264-3.521	1.265	0.302-5.302
>50	5	8.3	1.619	0.531-4.939	1.736	0.503-5.992
House with garden						
No	16	13.2	1.00	(Reference)	1.00	(Reference)
Yes	5	6.3	0.438	0.154-1.246	0.43	0.149-1.241
Work						
Not Exposed	11	11.0	1.00	(Reference)	1.00	(Reference)
Exposed	10	9.9	0.91	0.368-2.247	0.872	0.313-2.430

* OR-1 and 95%CI-1, crude ORs and CIs from univariable models.

** OR-2 and 95%CI-2, adjusted ORs and CIs from multivariable unconditional regression model containing all variables shown in the table.

(7), that TOS virus is the leading cause of human cases of acute meningitis and meningoencephalitis in central Italy, and that WN virus circulation is reported in northern, southern and central Italy (4, 5, 6, 18), we initiated a study intended to assess the potential relationships between arboviruses exposure and occupation.

The study of the prevalence of antibodies to WN, USU, TBE and TOS was carried out in a geographical area where outbreaks caused by these viruses had been documented in the past (4, 34). The study showed no seropositivity for WN, USU and TBE viruses and approximately 10% of all subjects resulted previously infected by TOSv. The seroprevalence to TOSv did not increase with specific occupational risk factors, probably because all outdoor activities favor exposure to sandflies bites irrespective of whether these are work related or not. One limitation of our study is linked with the difficulty in recruiting a higher number of participants among the exposed workers. These workers are mainly seasonal, often foreigners and cultural and language barriers could have determined the scarce participation in the survey. The limited number of subjects may also be responsible for the underestimated seroprevalence of antibodies against the other viruses. Further studies should therefore be performed to gain more insight into the circulation of arboviruses in wider geographical areas, where documented cases have not yet been reported, since the majority of this arboviral infections are subclinical. Recent investigations have also indicated that the *Phlebovirus* diversity in the Mediterranean basin is higher than initially suspected and novel viruses were reported to circulate in central Italy (26), posing the need of promoting more extensive epidemiological surveys. Monitoring workers at risk represents an important strategy to implement the available vaccinations (TBE virus), to promote further preventive measures and precautionary interventions for insect vectors control.

Although the serological results of our investigation did not show a higher risk for exposed workers, surveys conducted on outdoor workers remain useful for both public and occupational health purposes, providing early warning on the emergence of arboviruses in specific regions and among particular individuals.

NO POTENTIAL CONFLICT OF INTEREST RELEVANT TO THIS ARTICLE WAS REPORTED BY THE AUTHORS

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