Seroprevalence survey of arboviruses in workers from Tuscany, Italy

MARIA ELENA REMOLI¹, CRISTIANO FIORENTINI¹, ANTONELLA MARCHI¹, SIMONA DI RENZII, NICOLETTA VONESCH², VITTORIA MARIA PERI², LUCIA BASTIANINI², SONIA ROSSI³, GIULIA BARTOCCINI³, MAYA LISSA KUTTAPASERY⁶, MARIA GRAZIA CIUFOLINI¹, PAOLA TOMAO²

¹ Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie e Immunomediate, Rome, Italy
² INAIL, Dipartimento di Medicina, Epidemiologia, Igiene del Lavoro e Ambientale, Monteporzio Catone (Rome), Italy
³ Azienda Sanitaria Locale, Dipartimento di Prevenzione, Grosseto, Italy

KEY WORDS: Toscana virus; West Nile virus; Tick-borne encephalitis virus; Usutu virus; occupational infections; seroprevalence

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 artrpopodi (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
**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 central 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 arbovirosis 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 neutralizing 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.
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 Phleboviruses 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.
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

References

1. Alkan C, Bichaud L, de Lamballerie X, et al: Sandfly-borne phleboviruses of Eurasia and Africa: epidemiology, genetic diversity, geographic range, control measures. Antiviral Res 2013; 100: 54-74
2. Amaro F, Ciufolini MG, Venturi G, et al: Diagnóstico laboratorial de flebovírus (Virus Toscana). Acta Med Port 2007; 20: 341-345
3. Amicizia D, Domnick A, Panatto D, et al: Epidemiology of tick-borne encephalitis (TBE) in Europe and its prevention by available vaccines. Hum Vacc Immunother 2013; 9: 1163-1171
4. Autorino GL, Battisti A, Deubel V, et al: West Nile virus Epidemic in Horses, Tuscany Region, Italy. Emerg Infect Diseases 2002; 8: 1372-1377
5. Bagnarelli P, Marinelli K, Trotta D, et al: Human case of autochthonous West Nile virus lineage 2 infection in Italy, September 2011. Euro Surveill 2011; 16 (43): pii= 20002
6. Barzon L, Pacenti M, Franchin E, et al: Whole genome sequencing and phylogenetic analysis of West Nile virus lineage 1 and lineage 2 from human cases of infection, Italy, August 2013. Euro Surveill 2013; 18 (38). Erratum in: Euro Surveill 2013; 18 (40): pii= 20597
7. Beltrame A, Cruciani B, Ruscio M, et al: Tick-borne encephalitis in Friuli Venezia Giulia, Northeastern Italy. Infection 2005; 33: 158-159
8. Braitto A, Corbisorio R, Corradini S, et al: Evidence of Toscana virus infections without central nervous system involvement: A serological study. Eur J Epidemiol 1997; 13: 761-764
9. Calistani P, Giovannini A, Hubalek Z, et al: Epidemiology of West Nile in Europe and in the mediterranean basin. Open Virol J 2010; 4: 29-37
10. Cavrini F, Gaibani P, Longo G, et al: Usutu virus infection in a patient who underwent orthotopic liver transplantation, Italy, August-September 2009. Euro Surveill 2009; 4: 19448
11. Charrel RN, Bichaud L, de Lamballerie X: Emergence of Toscana virus in the mediterranean area. World J Virol 2012; 1: 135-141. doi: 10.5501/wjv.v1.i5.135
12. Chinikar S, Shah-Hosseini N, Mostafavi E, et al: Seroprevalence of West Nile virus in Iran. Vector Borne Zoonotic Dis 2013; 13: 586-589
13. Circolari del Ministero della Salute: Sorveglianza dei casi umani di Chikungunya, Dengue, West Nile Disease ed altre arbovirosi e valutazione del rischio di trasmissione in Italia – 2015-2017. http://www.salute.gov.it/
portale/temi/p2_6.jsp?lingua=italiano&id=4574&area=Malattie%20infettive&submenu=altremalattie

14. Colomba C, Saporito L, Ciufolini MG, et al: Prevalence of Toscana sandfly fever virus antibodies in neurological patients and control subjects in Sicily. New Microbiol 2012; 35: 161-165
15. D’Ovidio MC, Venturi G, Fiorentini C, et al: Occupational risk associated with Toscana virus infection in Tuscany, Italy. Occup Med 2008; 58: 540-544
16. Dobler G, Gniel D, Petermann R, et al: Epidemiology and distribution of tick-borne encephalitis. Wien Med Wochenschr. 2012; 162: 230-238
17. Epicentro (17 Novembre 2016), Rapporto N. 11. http://www.epicentro.iss.it/problemi/westNile/bollettino/WN_News_2016_11.pdf
18. Gaibani P, Pierro A, Lunghi G, et al: Seroprevalence of West Nile virus antibodies in blood donors living in the metropolitan area of Milan, Italy, 2009-2011. New Microbiol 2013; 36: 81-83
19. Günther G, Haglund M: Tick-borne encephalopathies: epidemiology, diagnosis, treatment and prevention. CNS Drugs 2005; 19: 1009-1032
20. Heinz FX, Holzmann H, Essl A, et al: Field effectiveness of vaccination against tick-borne encephalitis. Vaccine 2007; 25: 7559-7567
21. Lehrer AT, Holbrook MR: Tick-borne Encephalitis Vaccines. J Bioterror Biodef 2011; 2011(Suppl 1): 3
22. Liang G, Gao X, Gould EA: Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control. Emerg Microbes Infect 2015; 4: e18; doi: 10.1038/emi.2015.18
23. Llopis IV, Rossi L, Di Gennaro A, et al: Further circulation of West Nile and Usutu viruses in wild birds in Italy. Infect Genet Evol 2015; 32: 292-297. doi: 10.1016/j.meegid.2015.03.024.
24. Monaco F, Savini G, Calisti P, et al: 2009 West Nile disease epidemic in Italy: first evidence of overwintering in Western Europe? Res Vet Sci 2011; 91: 321-332
25. Pecorari M, Longo G, Gennari W, et al: First human case of Usutu virus neuroinvasive infection, Italy, August -September 2009. Euro Surveill 2009; 14: 19446
26. Remoli ME, Fortuna C, Marchi A, et al: Viral isolates of a novel putative phlebovirus in the Marche Region of Italy. Am J Trop Med Hyg 2014. doi: 10.4269/ajtmh.13-0457
27. Rizzo C, Salcuni P, Nicoletti L, et al: Epidemiological surveillance of West Nile neuroinvasive diseases in Italy, 2008 to 2011. Euro Surveill 2012; 17 (20): pii:20172
28. Savini G, Monaco F, Terregino C, et al: Usutu virus in Italy, an emergence or a silent infection? Vet Microbiol 2011; 151: 264-274
29. Savini G, Puggioni G, Di Gennaro A, et al: West Nile virus lineage 2 in Sardinian wild birds in 2012: a further threat to public health. Epidemiol Infect 2013; 141: 2313-2316
30. Smithburn KC, Hughes TP, Burke AW, Paul JH: A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med 1940; 2: 471-472
31. Tomao P, Ciceroni L, D’Ovidio MC, et al: Prevalence and incidence of antibodies to Borrelia burgdorferi and to tick-borne encephalitis virus in agricultural and forestry workers from Tuscany, Italy. Eur J Clin Microbiol Infect Dis 2005; 24: 457-463
32. Usama A, Jing Y, Xindi R, et al: Usutu Virus: An Emerging Flavivirus in Europe. Viruses 2015; 7: 219-238
33. Venturi G, Madeddu G, Rezza G, et al: Detection of Toscana virus central nervous system infections in Sardinia Island, Italy. J Clin Virol 2007; 40: 90-91
34. Verani P, Ciufolini MG, Nicoletti L: Arbovirus surveillance in Italy. Parassitologia 1995; 37: 105-108