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ORIGINAL ARTICLE

Seroprevalence study on the diffusion of the West Nile Virus among blood donors, healthcare workers, jockeys, grooms and fowlers, veterinary surgeons and hunters in Messina (Italy)

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Key words

Antibodies • WNV surveillance

Summary

Introduction. *West Nile virus (WNV) is a mosquito-transmitted flavivirus widely distributed in Africa, Middle East, Asia, Southern Europe and in 1999 was first identified in the United States as a cause of disease in New York City. It mainly circulates among birds, but can infect many species of mammals. Epidemics can occur in rural as well as urban areas.*
Methods. *1,280 sera were collected during 2006 from 80 stable workers, as jockey and grooms, 100 fowlers, 500 blood donors, 600 healthcare workers, 100 veterinary surgeons and 100 hunters in the Messina province to evaluate the prevalence of the WNV infection, by ELISA test, in relation to risk exposure or not.*
Results. *None of the 1280 subjects examined has shown positive for antibodies anti WN virus.*
Conclusion. *Among the strategies of control and surveillance, finally, in our opinion, are and will be indispensable the programs of continuous antibody survey in all the risk categories and in the general population in order to succeed to preview which effects could have the presence of infections from WNV, also imported from other zones where the virus is constantly present, in future and which it could be the impact of geographic factors on the epidemic spread of the disease.*

Introduction

West Nile Virus (WNV), mosquito-borne *Flavivirus* belonging to the Japanese encephalitis antigenic complex in the family *Flaviviridae*, is the most widespread arbovirus in the world [1-3] and in the Western Hemisphere it was detected for the first time in 1999 during an outbreak of encephalitis in New York City [4, 5]. WNV is transmitted primarily by the bite of infected mosquitoes that acquire the virus by feeding on infected birds [6-8]. Data on the incidence of WNV in most of the world are not readily available [9]. The ecologic aspects of WNV infection, involving mosquitoes, birds, and humans, were first described in the 1950s in Egypt [8, 10-12]. The agent circulates in nature through continuous enzootic transmission cycles between *Culicinae* mosquitoes and avian vertebrate hosts and may be introduced into a new territory by migratory birds. Humans and horses are considered incidental hosts [13, 14]. However, an urban cycle with virus amplification by continuous transmission between birds and vectors, and incidentally, humans, has been recently described in Romania where a few cases in the humans are noticed every year and in France where recent WN infections have been detected in monitored sentinel birds in 2001 and 2002 [15-18]. WNV transmission has been reported in other European Regions such as Portugal or United Kingdom where the virus apparently circulates but doesn't appear to present an imminent threat to human or animals. Many studies were done about the prevalence in different countries of the Old World: Dutchland, Spain, Netherlands but no evidence of WNV infection has been found in humans or horses. In Czech Republic an imported human case of WN fever (WNF) was recorded in 2002. Nine similar cases of WNF imported from the USA have already been reported in France, Denmark, Netherlands and Germany.

Other outbreaks happened in the last decades in the Middle East, Africa, India, parts of Asia, Australia (in the form of Kunjin virus, a subtype of WNV), North America, and parts of Central America and Caribbean, in Algeria in 1994, Morocco in 1996, Tunisia in 1997 and 2003, Romania in 1996 through 2000, the Czech Republic in 1997, Israel in 1999 and 2000, Russia in 1999 through 2001, and France in 2003 [9, 18-21]. Enzootics involving horses were reported in Morocco in 1996 and 2003, Italy in 1998, Israel in 2000, and Southern France in 2000, 2003, and 2004 [18, 22]. Since the virus was detected in New York from 1999 through 2004, 16,706 cases have been reported to the Centers for Disease Control and Prevention (CDC); 7,096 of these were classified as neuroinvasive disease, 9,268 as West Nile Fever (WNF), and 342 had other or unspecified clinical presentation (reported through June 8, 2005); the proportion of total cases reported that are neuroinvasive disease is actually higher than what is believed to occur naturally

since neuroinvasive disease is more likely to be reported than WNV or asymptomatic infection. The incidence of WNV disease is seasonal in the temperate zones of North America, Europe, and the Mediterranean Basin, with peak activity from July through October [23, 24]. Transmission of WNV in Southern Africa and of Kunjin virus in Australia increases in the early months of the year after heavy spring and summer rainfall [25, 26]. The most important risk factor for acquiring WNV infection is exposure to infected mosquitoes [27]. In Romania the risk for WNV infection was higher among persons with mosquitoes in their homes and with flooded basements [28]. An analysis of the locations of WNV disease cases during the 1999 outbreak in New York found that cases were clustered in an area with higher vegetation cover, indicating favourable mosquito habitat [29]. Human disease cases tended to occur in areas with more vegetation, older housing, lower population density, predominance of older Caucasian residents, and proximity to dead birds, but the effects of these variables were influenced by differences in mosquito abatement efforts [30]. Risk factors for infection not related to mosquito exposure include receiving blood transfusions or organ donations, maternal infection during pregnancy or breastfeeding, and occupational exposure to the virus [21, 31, 32]. Statistically, a personal risk of contracting West Nile is low, and less than 1% of those infected develop serious illness from the virus. Those at highest risk for serious illness are the elderly and those with lowered immune systems. However, people of all ages can develop serious illness, so it is important for everyone, as mentioned before, to protect themselves from mosquito bites to minimize the risk of infection. Infectious blood components with low concentrations of WNV may escape current screening tests (CDC 2004) [28]. One instance of possible WNV transmission through dialysis has been reported [33]. WNV transmission through organ transplantation was also first described during the 2002 epidemic [31], moreover, WNV infection has been occupationally acquired by laboratory workers through percutaneous inoculation and possibly through aerosol exposure [34, 35]. Currently there is not specific drug treatment or vaccine against the infection even if there are many experiments to produce a specific vaccine [36-38]. About Italian situation, until 1998 no clinical or serological confirmed case of WN disease was ever indicated on national territory [39]. However, the doubt that a similar Flavivirus WN circulated in Italy was been marked in the past by serological positives verification in human patients and in sheep. The fact that the isolated antibodies were never neutralizing made the experts think that it was a similar virus but not the same. Virus isolation attempts were always negative. The first official communication of WN encephalitis in horses bred in Tuscany dates back to September 1998. The illness began with ataxy to then degenerate in paraplegy, tetraplegy, coma and death. Usually there was no fever. The heald subjects regained normal functions in different times spends. On September 20 Tuscany began a health control that included: the prohibition of moving the horses in or out of the area, careful disinfection of the stables where deaths occurred. During the outbreak no human case was detected while 3.1% of people working in these environments showed IgG prevalence. On the basis of the explained epidemiological data, it is more evident that the information about the diffusion of this infection in Italy are very poor, therefore, in the present paper a seroepidemiological study was conducted to define the prevalence of WN virus infection among blood donors, jockeys, grooms, fowlers, veterinary surgeons and hunters in the Messina (Italy) area.

Materials and methods

Clinical samples

1,280 serum specimens were collected during 2006 from 80 stable workers, as jockey and grooms, 100 fowlers, 500 blood donors and 600 health workers, 100 veterinary surgeons, and 100 hunters in the Messina province to evaluate the infection prevalence in relation to risk exposure or not. The samples were stored at -80 °C until Elisa test commenced. IgG were screened to evaluate the circulation of the infection into the population in the last two years because they could be found after three weeks from infection for at least 500 days. On the contrary IgM disappear after two or three months from the beginning of the disease. Also, many samples from a non endemic zone were tested and it would have been really difficult to found IgM in samples sera. In case of positivity "Plaque Reduction Neutralization Test" will be used to confirm the results.

WNV IgG enzyme immunoassays

All the samples were tested by IgG antibodies against WNV by the Focus Technologies WNV IgG DxSelect ELISA where the polystyrene microwells are coated with recombinant West Nile Virus antigen. Testing was performed according to the manufacturers' instructions: briefly, after bringing all reagents and samples to room temperature, we diluted the samples 1:101 in Sample Diluent and filled wells with 1X Wash Buffer Solution allowing to soak for 5 minutes. In the second step we dispensed 100 µl of the sample diluent into the "blank" wells, 100 µl of each diluted specimen, control or calibrator into the appropriate wells and 100 µl of each sample in the remaining 90 wells and incubated for 60 minutes at room temperature after covering plates or placed in a humid chamber. The third step consisted in decanting and washing the plaque three times and dispensing 100 µl conjugate to all wells and incubating for thirty minutes at room temperature. After we decanted and washed three times we added 100 µl of Substrate reagent to all wells and incubated for 10 minutes. Lastly we added 100 µl of Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells the colour should have changed from blue to yellow. The last step was the reading at $\lambda = 450$ nm after zeroing the instrument on the blank wells.

Results and discussion

None of the 1,280 subjects examined for antibodies anti WN virus positive resulted even though different categories of subjects (blood donors, operating sanitary, jockey and horse grooms, fowlers, veterinary surgeon, hunters), who for various reasons could be exposed to the infection, were examined. The indisputable data have demonstrated the total absence of contact with the virus, supporting hypothesis that the outbreak of equine encephalomyelitis due to West Nile Virus occurred in the Tuscany region of Central Italy [20] represented a circumscribed phenomenon. Even if, for this purpose, it must be remembered that whereas the first isolates of WNV were obtained from 1998 in the mentioned Region, antibodies were detected previously in human sera from Northern and Central Italy (1967) and in migratory birds in Northern Italy (1973) during the autumn migration suggesting that WNV, or a closely related arbovirus, has been circulating in Italy for decades [39]. Our data only confirms the total absence of human cases in this last decade in our territory, as far as the South of Italy and Sicily. This is a superimposable situation to the other European countries where this kind of study were conducted even though sometimes with different analysis methodologies as for example in Dutch blood donors.

However, this could be related to the fact that Italy, owing to the outbreak of 1998, has adopted a plan of national surveillance in veterinary circles, through the 04/04/2002 Ministerial Ordinance (MO). Then (as a result of some seroconversions in horses) the MO has been repeated from the year 2004 until December 31, 2007 with Decree of the Health Ministry (07/13/2005). The areas of study were characterized in the plan, spread over all the National territory, that for their climatic characteristics (humid, swampy, rich zones of aquatic birds and high density of bug carriers) can be considered suitable for the virus introduction and propagation. Inside these areas the ASL Veterinary Services put into effect the surveillance plan that is based essentially in the search of the virus in the birds found dead and in the bugs, while in the horses and groups of chickens located in vicinity of the zones of greater concentration of wild bird migration (net of "chickens lookout"), antibodies against the agent of the disease are found. All this has allowed the activation of a system of alert necessary to express eventual previsions in case of virus presence in the surveillance area. The surveillance in the human population, previewed in the same 04/04/2002 MO, is defined in the Circular of the Ministry of the Health of the 18/09/2002. The prevention of the disease in man is essentially based on the fight against mosquitos (insecticide, reclamation acclimatizes them) and in the application of protection measures against the bugs, while for the animals, apart from the fight against the carriers, a vaccine is available (not in Italy).

Conclusion

The present study, in conclusion, can be included, in the program of control and surveillance of the infections spread from WNV to our Country and supplies an effective answer in considering the absence of risk of infection in the categories examined. The obtained results have not suggested abandoning the current effected strategy but indeed they want to give a contribution to the increment and planning of continuous participation turned to the intensification of the efforts to reduce the places of reproduction of the mosquitos and to increase the application of larvicides in order to kill the grubs in those areas in which the virus could be found, represented by tinning water zones that cannot be completely drained. Other measures of prevention to intensify would be to regularly subject mosquitos and birds to the test for the WNV in spring and summer and in the case in which the virus is found to levels that indicate a threat for human health aimed application of pesticides to prevent the infection among the population. In any case, it is undoubted that in Europe in the past two decades there have been a significant number outbreaks in several countries. However, very little is known of the ecology and natural history of WN virus transmission in Europe and most WN outbreaks in humans and animals remain unpredictable and difficult to control. For this reason, among the strategies of control and surveillance, in our opinion, the programs of antibodies survey are and will be useful in all the risk categories and in the general population in order to predict which effects could have the presence of infections from WNV, also imported from other zones where the virus is constantly present, in future and which could be the geographic impact factors on the epidemic spread of the disease

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