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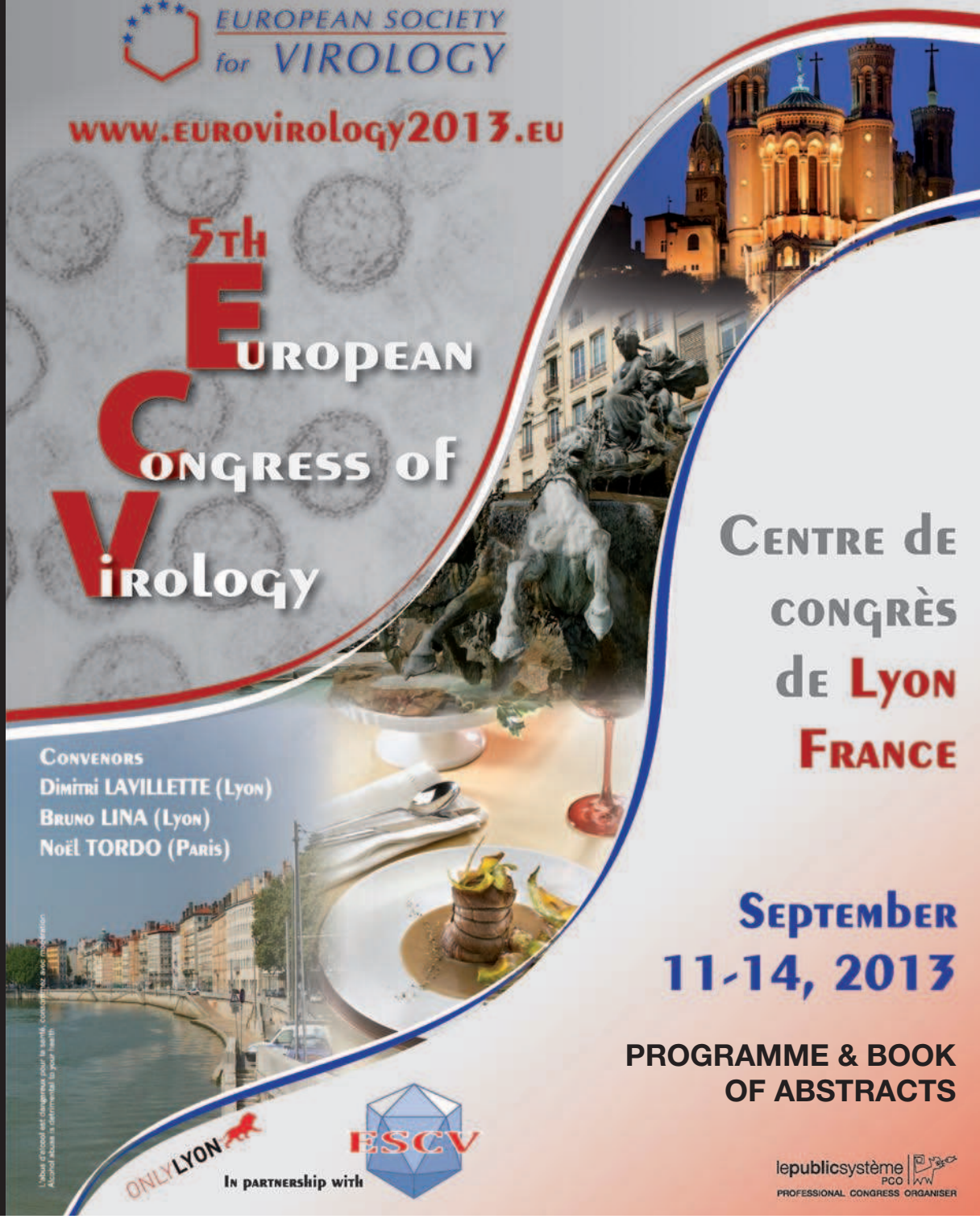

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and PCR fragments were sequenced directly. The viral non coding ends were determined with the SMART RACE method (Matz 2003). Phylogenetic analyses confirmed the relationship of ILEV to the Bunyamwera serogroup of orthobunyaviruses. ILEV segments S and L exhibited highest nucleotide sequence identities with Bunyamwera virus (86% for S, 80% for L) and Ngari virus (87% for S, 81% for L). The M segment of ILEV however shared only a maximum of 70% sequence identity with several members of the Bunyamwera serogroup (e.g. Ngari virus, Northway virus, Cache Valley virus). Notably, all three ILEV segments displayed nucleotide identities of 99% with the partially available sequences of Mboke virus, which would suggest that Mboke virus is an ILEV isolate.

REF 290

Monitoring of Phlebotomus trasmitted viruses activity in Marche region, Italy

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Phlebotomine sand flies are known to transmit leishmaniases, bacteria and viruses that affect humans and animals in many countries worldwide. Sand flies are widely distributed in all countries around the Mediterranean, and thus human populations in this area are exposed to sand fly transmitted diseases. Sand fly borne viruses are mainly belonging to the genus Phlebovirus, the Vesiculovirus and the Orbivirus. The phleboviruses transmitted by sand flies are a major cause of meningitis, encephalitis and febrile illnesses. To monitor the stability of the Phlebotomus transmitted viruses "foci" and to investigate about the possible circulation of different viral variants, surveillance activity was carried out at the end of summer 2012 in a known Italian Phlebotomus trasmetted viruses historical focus in Fermo commune (Marche region) (Ciufolini. et al., 1992, Parassitologia, 34, Suppl. 1: 7 8) monospecific for Phlebotomus perfiliewi. Nine hundred sand fly specimens (480 females (F) and 420 males (M)) were collected, pooled and processed both by nested PCR, using generic primers for the genus Phlebovirus, and by inoculation in VERO cells for virus isolation. Seven pools resulted positive to RT PCR analysis (5 F and 2 M). Four of them showed also a CPE in VERO cells. Preliminary study about the polimerase gene (L segment) sequence analysis showed the confirmation of the presence of Toscana virus and the possible circulation of a new Phlebovirus. Analysis is in progress to characterize the complete genome of the new isolates. However our data confirm the stability of Phlebovirus focus in Fermo province. A comparison between historical data on virus genotypic stability have been planned. This work was supported by EDENext, a collaborative project of the 7th FP (2011 2014) funded by the European Commission under the DG Health; Contract Number: 261504

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West Nile Virus interactions with viruses usually infecting the most probable West Nile Virus vectors

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Pathogenic arboviruses have been the focus of many investigations. However, in the vectors that carry them, other viruses are also present. Interactions between them have been less studied. One such example is that of the flaviviruses. This genus contains important public health challenges such as West Nile. In recent years, many new flaviviruses have been described, most of them belonging to the so called "mosquito only flaviviruses" group. Other new mosquito only viruses such as birna, alpha, mesoni or negeviruses have also been recently described. With the aim of initiating an investigation about the interactions between viruses circulating in Spain and Italy, we have conducted systematic virus isolation in C636 cells from mosquito pools. When cythopatic effect was detected, isolates were analyzed by electron microscopy, consensus PCR and/or sequencing (by generic primers and high throughput sequencing systems). Sixty six pools of mosquitoes of the species Culex pipiens, Cx. modestus, Cx perexiguus, Ochlerotatus caspius and Aedes albopictus were collected in Spain. Eighteen viruses were identified (11 flavi, 2 reo, 3 parvo (1 denso), 1 ortorthobunya and 1 negevirus). Fifty two pools of the species Cx pipiens, Cx modestus and Ae albopictus were collected in Italy. Fourteen viruses were identified (6 reo, 4 rhabdo 5 flavi, 1 birna, 2 parvo and 2 negeviruses). Most of the positive pools contained two or three viruses. Thus, it is possible that a mosquito could be infected with more than one agent. Possible implication of these will be discussed.

REF 292

Wild bird surveillance of West Nile virus in an endemic area

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West Nile virus (WNV) is maintained in nature in a cycle between Culex mosquitoes and wild birds. To better understand the complex mechanism of WNV maintenance and transmission in an endemic area, a serosurvey and RT qPCR were carried out. Wild birds of 25 different species were sampled from October 2011 to January 2012 and from May to July 2012 near Tulcea, in the Danube Delta. Four hundred forty five sera samples were screened for WNV Ab with the INGEZIM ELISA and positive sera were conducted to a plaque reduction neutralization test (PRNT). Oral and cloacal swabs of 350 birds (pooled per 4 birds) and different organ samples and swabs of 83 birds (pooled per bird) were tested for presence of WNV nucleic acid by use of RT qPCR. Sixty out of 445 sera were tested positive in the INGEZIM ELISA whereas only 19 sera were verified with the PRNT. Antibodies specific to WNV were detected in birds of the family Passeridae (4/178), Corvidae (6/76), Sylvidae (2/28), Lanidae (1/28), Orolidae (3/3), Upupidae (2/2), and Ardeidae (1/1). No WNV nucleic acid was detected in any of the wild birds sampled. Our serological and virological data were not indicative of any acute/persistent infections or WNV shedding in the investigated wild birds. However, considering the great biodiversity and large amounts of wild birds in this endemic area our sample size was restricted. Moreover, only a limited number of birds may be involved in the WNV transmission cycle. On the basis of our results, we assume that there was limited WNV circulation in wild birds in Tulcea in winter 2011/12 and summer 2012.