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Short communication

Occurrence of a case of influenza A(H_1N_1)pdm09 and B co-infection during the epidemic season 2012–2013

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ABSTRACT

We report the detection of one case of co-infection with influenza A(H_1N_1)pdm09 and B, occurred during the 2012–2013 influenza season in Sicily. The dual infection was identified in a 18-year-old boy, who was not covered by specific vaccination and who had no other pre-existing risk factors. He presented classical symptoms of influenza-like illness developing no respiratory complications. A(H_1N_1)pdm09 viral concentration was initially about 10-fold higher than B virus, whereas its clearance was more rapidly achieved than in the case of B virus infection. Although influenza co-infection appears to be a rare event, a continued influenza surveillance activity is recommended, in order to evaluate diversity and evolution, but also to support public health prevention measures.

1. Introduction

In Sicily, epidemiological and virological surveillance of influenza is annually conducted as part of the nationwide active sentinel surveillance coordinated by the National Institute of Health (Istituto Superiore di Sanità – ISS) in Rome.

In this context, the incidence of medically attended influenza-like illness (ILI) is monitored by a group of sentinel practitioners (general practitioners and paediatricians) covering about 2% of the general population, co-operated by a limited number of hospital physicians, in order to collect, each influenza season, combined clinical and virological information on circulating viral strains from week 46 to week 17 of the following year.

It is well documented that influenza epidemics are commonly caused by type A and B viruses, differing year by year because of the alternance or co-circulation of types and subtypes such as A(H_1N_1) and A(H_3N_2), and offering the opportunity for genetic reassortment as a consequence of possible co-infection in human population (Ju et al., 2010).

In contrast to the previous 3-year period, in which influenza A clearly predominated, national data collected during 2012–2013 depicted an epidemiological scenario characterized by a significant co-circulation of influenza type A and B viruses (42.3% *vs.* 57.7%, respectively) [http://www.iss.it/binary/fluv/cont/Agg.Vir_2_5_13.pdf]. Although co-infection with both influenza A and B viruses appears to be a rare event (Falchi et al., 2008; Almajhdi and Ali, 2013), in this study we describe the detection of a single case of co-infection caused by influenza A(H_1N_1)pdm09 and B viruses in Sicily during the 2012–2013 influenza epidemic.

2. Materials and methods

From November 2012 to April 2013, 321 nasopharyngeal swabs (Virocult, MWE Medical Wire, UK) were collected from patients (53.1% males; median age 19 years, IQR 45 years) suffering from an influenza-like illness and then analyzed at the Regional Reference Laboratory for Influenza Surveillance and Molecular Characterization.

According to the Italian influenza network (INFLUNET) operating protocol [http://www.iss.it/binary/iflu/cont/Prot14.pdf], all patients presented with typical symptoms of influenza, including sudden onset of fever (⩾38 °C) plus at least one respiratory symptom (cough, sore throat, rhinitis) and at least one systemic symptom (headache, malaise, chills, asthenia). After getting an informed consent from each patient or parents/guardians, all swabs were collected and then immediately refrigerated at 4 °C

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for transport. Upon arrival, specimens were eluted in Dulbecco's Modified Eagle Medium (DMEM) and divided into aliquots for molecular assays or stored at −80 °C for further analyses.

Extraction of viral RNA from 140 μL of clinical samples was conducted using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's guidelines.

Detection of influenza A and B viruses, and housekeeping gene RNase P was performed using the Superscript® III Platinum One-Step qRT-PCR Kit (Life Technologies, Carlsbard, USA) on ABI Prism 7000 Real-Time PCR instrument (Applied Biosystems, Foster City, CA) using specific primer and probe sets (Table S1). Positive and negative controls were included in each real-time RT-PCR run.

The viral co-infection was confirmed using MDCK cells for influenza virus propagation, performing both real-time RT-PCR and one-step PCR amplifications on culture supernatant. Hemoagglutinin (HA) and neuraminidase (NA) nucleotide sequences were obtained from both A(H_1N_1)pdm09 and B co-infecting viruses by direct sequencing (Fig. S1), using the BigDye terminator chemistry version 3.1 (Applied Biosystems, Foster City, CA) according to the instructions of the manufacturer.

Both HA and NA sequences were submitted to GenBank with the following accession numbers: KF700627, KF700628, KF700629, KF700630.

The present work was reviewed and approved by the institutional review board of the University Hospital "P. Giaccone" of Palermo (Sicily), health data were stored according to the Italian laws on privacy, and the research was conducted following the Helsinki declaration statements.

3. Results

In Sicily, the winter annual influenza outbreak began in January and ended in March 2013 (Fig. 1). Overall, 159 out of 321 (49.5%) samples tested positive for influenza, type A viruses were identified in 45 out of 159 positive samples (28.3%: A(H_1N_1)pdm09, $n = 42$; A(H_3N_2), $n = 2$; A-untypable, $n = 1$), whereas influenza B was detected in 113 out of 159 (71.1%); only one patient (0.6%) exhibited a co-infection with A(H_1N_1)pdm09 and B.

Of note, type B viruses prevailed during the first half of the epidemic season, whereas late in February a higher proportion of influenza A(H_1N_1)pdm09 infections became evident (Fig. 2). About one week before the breakpoint (52.6% type A and 47.4% type B, respectively), it was detected the only case of influenza type A and B co-infection here reported.

3.1. Clinical and virological data of co-infected patient

This dual infection was identified in a 18-year-old boy with no influenza vaccination coverage and no history of travelling out of Sicily during the winter season.

At the time of nasopharyngeal swabbing (February 25, 2013), he was at the third day of classical ILI symptoms (rapid onset of fever to 38.5–39.0 °C, myalgia, arthralgia, headache, and sore throat) together with tonsillar hypertrophy; no other pathologic signs were observed. The patient was treated with acetaminophen and, after 5 days, fever continued to be high, decreasing to 37.0 °C on March 5 (11 days from onset).

On March 7, fever totally disappeared, as well as headache, myalgia, and sore throat, while the tonsillar hypertrophy persisted. Finally, on March 8, the boy resumed his school activity.

After laboratory viral detection on February 25, in order to monitor the evolution of the dual infection and the kinetic of viral clearance, nasopharingeal swabbing was further performed until viral negativization (Table 1). Interestingly, although the concentration of A(H_1N_1)pdm09 virus was initially about 10-fold higher than B virus, as displayed by the real time cycle threshold (Ct) values (Ct = 31 *vs.* Ct = 34: A(H_1N_1)pdm09 and B, respectively), its clearance was more rapidly achieved than in the case of B virus infection, which persisted for about one-week more. The third nasopharyngeal specimen (March 5, 2013) was negative for both the viruses, as definitively confirmed with the fourth specimen collected on March 9, 2013 (Table 1).

4. Discussion

Dual infection with influenza viruses represents an infrequent event and, to date, only few papers have been published mainly reporting A(H_3N_2) and B co-infections (Shimada et al., 2006; Toda et al., 2006; Falchi et al., 2008; Eshaghi et al., 2009), with just

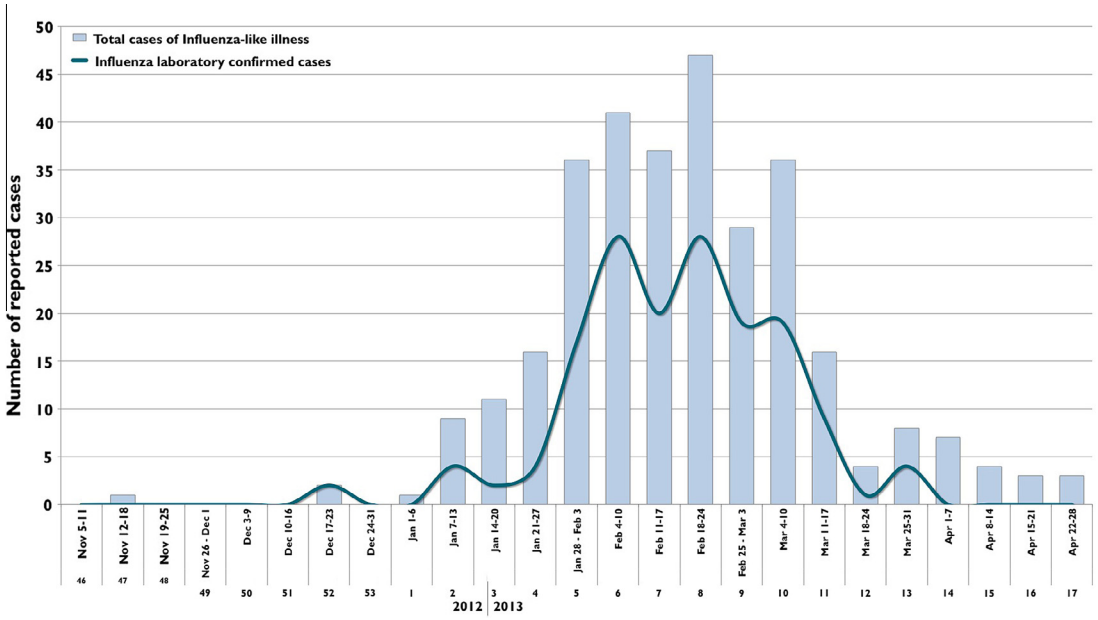

Fig. 1. Weekly distribution of influenza-like illness ($n = 321$) and influenza laboratory-confirmed ($n = 159$) cases in Sicily, November 2012–April 2013. The graph depicts the weekly cumulative number of influenza-like illness and influenza laboratory-confirmed cases reported during the surveillance season 2012–2013.

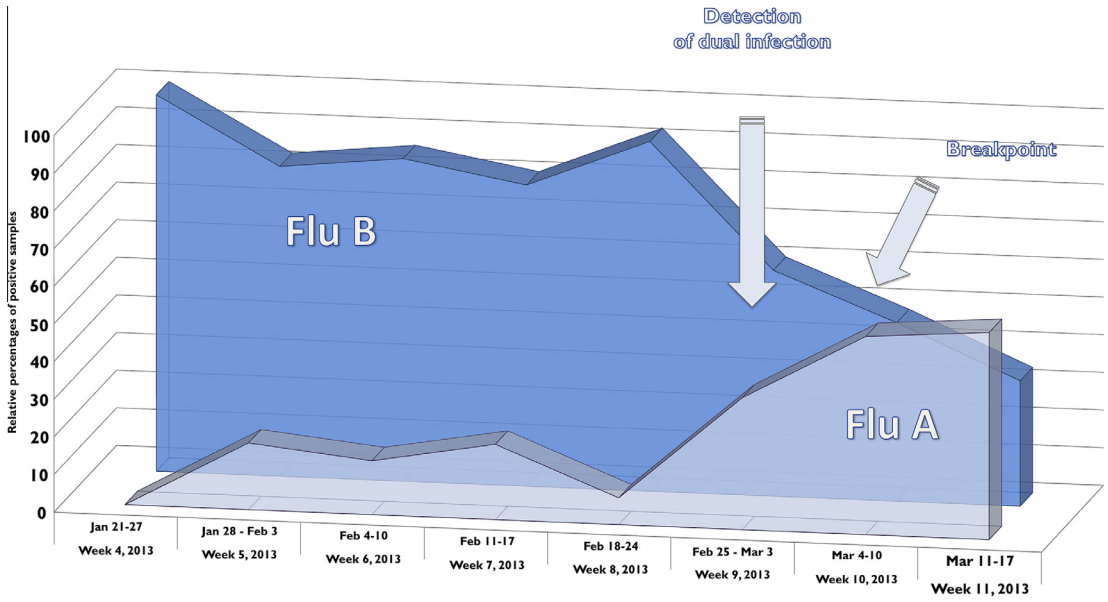

Fig. 2. Relative percentages of positive samples. Influenza type A or B % *vs.* Total %. The plot highlights the relative percentages of influenza type A or type B infections versus the overall percentage of influenza infections, for each week of virologic surveillance. Moreover, the graph shows the week of detection of the dual-infection.

Table 1
Real-time cycle threshold (Ct) values and kinetic of viral clearance in nasopharyngeal swabs.

Days of collection	Influenza viruses	
	Type A(H_1N_1)pdm09	Type B
February 25, 2013	Positive (Ct = 31)	Positive (Ct = 34)
March 4, 2013	Negative	Positive (Ct = 38)
March 5, 2013	Negative	Negative
March 9, 2013	Negative	Negative

one case of co-infection attributed to influenza virus A(H_1N_1) pdm09 and B (Calistri et al., 2011).

The factors that may be responsible for such virological event are not yet clear. In fact, dual infections have been documented among people of different age-groups and both in immunocompromised patients, thus suggesting a potential role of the immunological state of the host (Toda et al., 2006; Almajhdi and Ali, 2013), and in "healthy" patients without clinical complications (Shimada et al., 2006; Toda et al., 2006; Falchi et al., 2008; Ju et al., 2010), as also described in the present paper. Moreover, the fact that most clinical manifestations observed in dual infections are quite similar to those of single infections, characterized by self-limiting mild classical symptoms, does not support the hypothesis of an association between co-infection and severity of disease.

According to other authors (Shimada et al., 2006), different levels of influenza viral types were observed in the co-infected clinical sample, with a predominance of influenza type A, although in association with a more rapid viral clearance in respect to type B. Even though this finding may reflect different replication efficiency of influenza viruses (Shimada et al., 2006), the literature is poorly represented on this topic and contrasting results have also been recently reported (Calistri et al., 2011).

Finally, of note, our case of co-infection happened in an unusual epidemiological context characterized by a very high circulation of influenza B strains (71.1%), partially overlapped with an increasing spread of influenza A strains. This could have played a role in the occurence of dual infection but more data are needed to confirm this observation.

In conclusion, although rare, the detection of influenza co-infection could still represent an underestimated phenomenon, either in terms of social impact or clinical implications, and it surely contributes as a potential source of multiple viral transmission to susceptible individuals.

Of course, the main limit of the present study is the lack of consecutive samples showing evidence of dual infection, which certainly would have better characterized our findings.

Nevertheless, these considerations suggest a continued surveillance activity of monitoring distribution, prevalences, and molecular characteristics of circulating influenza strains, in order to evaluate their diversity and evolution, but also to develop and/or optimize public health prevention and control strategies.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Conceived and designed the study: F.T., F.V. Collected clinical and epidemiological data: F.T., C.M.M., F.M. Analysed data: F.T., E.A. Wrote the paper: F.T., F.V.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.01.032.

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