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Short Report

Characterization of an influenza B virus isolated from a fatal case of myocarditis in a pediatric patient in Italy

Simona Puzelli [a,*,1], Marzia Facchini [a,1], Sara Piacentini [a], Giuseppina Di Mario [a], Maria Eugenia Colucci [b], Laura Calzoletti [a], Concetta Fabiani [a], Angela Di Martino [a], Licia Veronesi [b], Giacomo Biasucci [b,c], Mauro Codeluppi [d], Giuliana Lo Cascio [e], Roberta Schiavo [e], Alessandra Rampini [f], Paola Affanni [b], Anna Teresa Palamara [a], Paola Stefanelli [a]

[a] Department of Infectious Diseases, Istituto Superiore di Sanità (ISS), Rome, Italy
[b] Department of Medicine and Surgery, University of Parma, Parma, Italy
[c] Pediatrics & Neonatology Unit, Guglielmo da Saliceto Hospital, Piacenza, Italy
[d] Infectious Diseases Unit, Guglielmo da Saliceto Hospital, Piacenza, Italy
[e] Microbiology Unit, Guglielmo da Saliceto Hospital, Piacenza, Italy
[f] Hygiene and Public Health Unit, Local Health Authority, Piacenza, Italy

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ABSTRACT

Influenza B is one of the infective agents that can cause rapid and fatal myocarditis in children. Here, we describe a fatal case of myocarditis in a previously healthy child, after infection with an influenza B/Victoria-lineage virus during the 2022–23 epidemic season in Italy. Influenza B virus was isolated also in a second case, a younger family member showing only a mild influenza-like illness. Genotypic and phenotypic analyses have been performed on both virus samples and results showed that HA1 sequences were identical and genetically and antigenically related to other B viruses circulating in 2022–23 season in Italy. However, a D129N substitution was found in the receptor binding domain of the HA of the two viruses, not detected in other circulating viruses in Italy but only in a proportion of those circulating in other European countries. Phenotypic analyses assessed the susceptibility towards either neuraminidase inhibitors and baloxavir. Annual influenza vaccination remains one of the best interventions to prevent complications such as myocarditis, particularly in children.

Introduction

Influenza is generally considered an infrequent cause of myocarditis, which may occur in up to 10% of all influenza cases, either in children or in adults [1–4]. The incidence of this severe complication during influenza epidemics may depend on the nature of circulating strains and rates may be higher during severe seasons [2]. Here, we report the characterization of an influenza B virus isolated in a child with a rapid fatal myocarditis outcome (case 1). A second mild case in the same family was also studied (case 2).

Case report

On 24th of March 2023, a 6-year-old girl (case 1) presented to the emergency unit with vomit, asthenia and abdominalgia. At the hospital, the case was afebrile with a hearth rate of 96 beats/min. The child did not receive vaccination against either flu or COVID-19. No history of travels, serious illnesses or allergies were described. Nevertheless, 3 months before admission the child suffered of a Streptococcal infection treated with antibiotics and it is also to underline that, the week before symptoms onset, the child attended a public event crowded with people coming from all over Europe.

Laboratory findings showed a troponin I level of 1416.6 ng/L (normal range-n.r.: < 12 ng/L), creatine kinase (CK) of 329 IU/L (n.r.: 0–172 IU/L) and CK MB of 15.1 ng/mL (n.r.: 0.6–6.3 ng/mL). An electrocardiogram (ECG) showed sinus rhythm and low electrocardiographic QRS voltage. Few hours after hospitalization, hearth

* Corresponding author.
E-mail address: simona.puzelli@iss.it (S. Puzelli).
[1] These authors contributed equally to this work and share first authorship.

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rate was 164 beats/min and the echocardiogram demonstrated diffuse ipokinesia, severely compromised pump function (ejection fraction: 20% max.) and mitral insufficiency. Antiviral therapy was not initiated due to the rapid worsening of clinical conditions. The patient was then transferred to an intensive pediatric care unit in another Hospital, for further management. During the transfer, intravenous adrenalin was administered. Cardiac arrest occurred rapidly and the child died during the night, 5 days after symptoms onset. Moreover, a 3-year-old girl (case 2), a family member related to case 1, showed mild symptoms of an influenza-like illness (ILI) and resulted positive to influenza B.

RT-PCR analysis of a nasopharyngeal swab collected after hospital admission was positive for influenza B and confirmed by the regional reference InfluNet laboratory in Parma. The sample was subsequently sent to the laboratory of the WHO National Influenza Centre at ISS (NIC) in Rome for isolation and further genotypic and phenotypic characterizations. NIC also received a nasopharyngeal swab collected on 27th March 2023 from case 2. Both influenza B positive samples, that formerly tested negative to a multiplex real-time PCR for SARS-CoV-2 and RSV (Panther Fusion™ SARS-CoV-2/Flu A/B/RSV Assay - Hologic), were grown in MDCK cells. The sample from the fatal case was also examined for the detection of specific Enterovirus 5′-UTR region by Real Time PCR analysis (RIDA®GENE Enterovirus, r-BioPharm) and resulted negative.

Phylogenetic analysis was performed on the HA1 portion of the hemagglutinin (HA) gene of the virus isolates and compared with other sequences from Italy and Europe, available on GISAID [Fig. 1]. The results showed that HA1 from case 1 and case 2 were identical (100% nt. identity) and belonged to the B/Victoria lineage, subgroup V1A.3a.2 (represented by the 2022–23 NH vaccine strain, B/Austria/1359417/2021); V1A.3a.2 clade was dominant among influenza B viruses circulating across Europe in the 2022–23 season. The phylogenetic tree also showed that the two viruses were genetically similar to other influenza B viruses circulating in Italy in the same period, including those from some mild cases from the same Region. No mutations of note were detected in the viral HA1, except for a D129N amino acid substitution found in both viruses, if compared to B/Austria/1359417/2021 [Table 1]; this amino acid is located in the receptor binding domain (RBD) of HA [5]. The above substitution was not detected in other B/Victoria viruses circulating in Italy during the season, but it was found in other B/Victoria viruses identified in EU in 2023 [Fig. 1]. With regard to neuraminidase (NA) genes, no significant mutations were detected and both viruses were susceptible to the NA inhibitors, oseltamivir and zanamivir [6]. Phenotypic evaluation, using the MUNANA fluorescence-based NA-enzyme inhibition assay [7], confirmed the susceptibility toward both drugs. Moreover, molecular analysis on the polymerase acidic protein (PA) gene did not identify markers associated with reduced susceptibility for baloxavir [8]. Antigenic characterization by hemagglutination inhibition (HI) assay [9], showed that the viruses isolated were well recognized by antiserum raised against B/Austria/1359417/2021.

Discussion

Influenza B viruses mostly cause mild to moderate respiratory illness in healthy children. However, some pulmonary and extrapulmonary complications, including myocarditis, have been reported to be associated with influenza B infection, particularly in children [10]. The viral infection may contribute to myocardial injury in different ways: it may involve a specific effect linked to the influenza virus infection (direct damage), systemic inflammatory response, or a combination of both [11]. However, the mechanisms leading to mortality are still unclear.

Hereby, we performed a characterization of an influenza B/Victoria virus associated with a fatal case of pediatric myocarditis and of the B/Victoria virus isolated from a family member with mild ILI. Phylogenetic analysis of the HA1 sequences, although similar to other influenza B viruses circulating in 2022–23 season in Italy, showed the presence of a common D129N amino acid substitution in the RBD region. This mutation was not detected in other B/Victoria viruses circulating in Italy, but was found in a proportion of those circulating in EU, retrieved from GISAID. The public event attended by the child the week before symptoms onset might explain the observed clustering of the two HA1 sequences with other EU strains [Fig. 1]. To our knowledge, there are not scientific evidences showing an association of this amino acid change with extrapulmonary complications. Moreover, it is not possible to exclude that the previous bacterial infection might have played a role in the fatal outcome. Due to the current evidences and to the open questions on viral genetic determinants of influenza B pathogenicity, host factors and environmental features, further studies should be carried out to better comprehend why some previously healthy patients recover without any residual injury, whereas others develop fatal outcome [1].

It should be mentioned that the 2022–23 season in Italy, as well as in Europe, has been characterized by a sustained influenza virus circulation, attributable to both type A and B viruses, with these latter prevailing from March 2023 onwards. On the other hand, the reduced influenza virus circulation during the preceding 2020–21 and 2021–22 seasons worldwide, after the onset of the COVID-19 pandemic, and the consequent limited exposure of the population to these viruses, may have had an impact on the population immunity to the currently circulating influenza viruses [12,13]. Severe cases of influenza B among children and adolescent, requiring critical intensive care were also reported in Sweden in March 2023 [12]. Annual influenza vaccination remains one of the main actions to prevent complications such as myocarditis, particularly in children.

Ethics approval and consent to participate

No specific ethical approval was required for this study. The analyzed samples were collected within routine influenza surveillance activities. However, informed consent has been obtained from the children's parents that consented to this report.

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Declaration of Competing Interest

The authors have no competing interests to declare.

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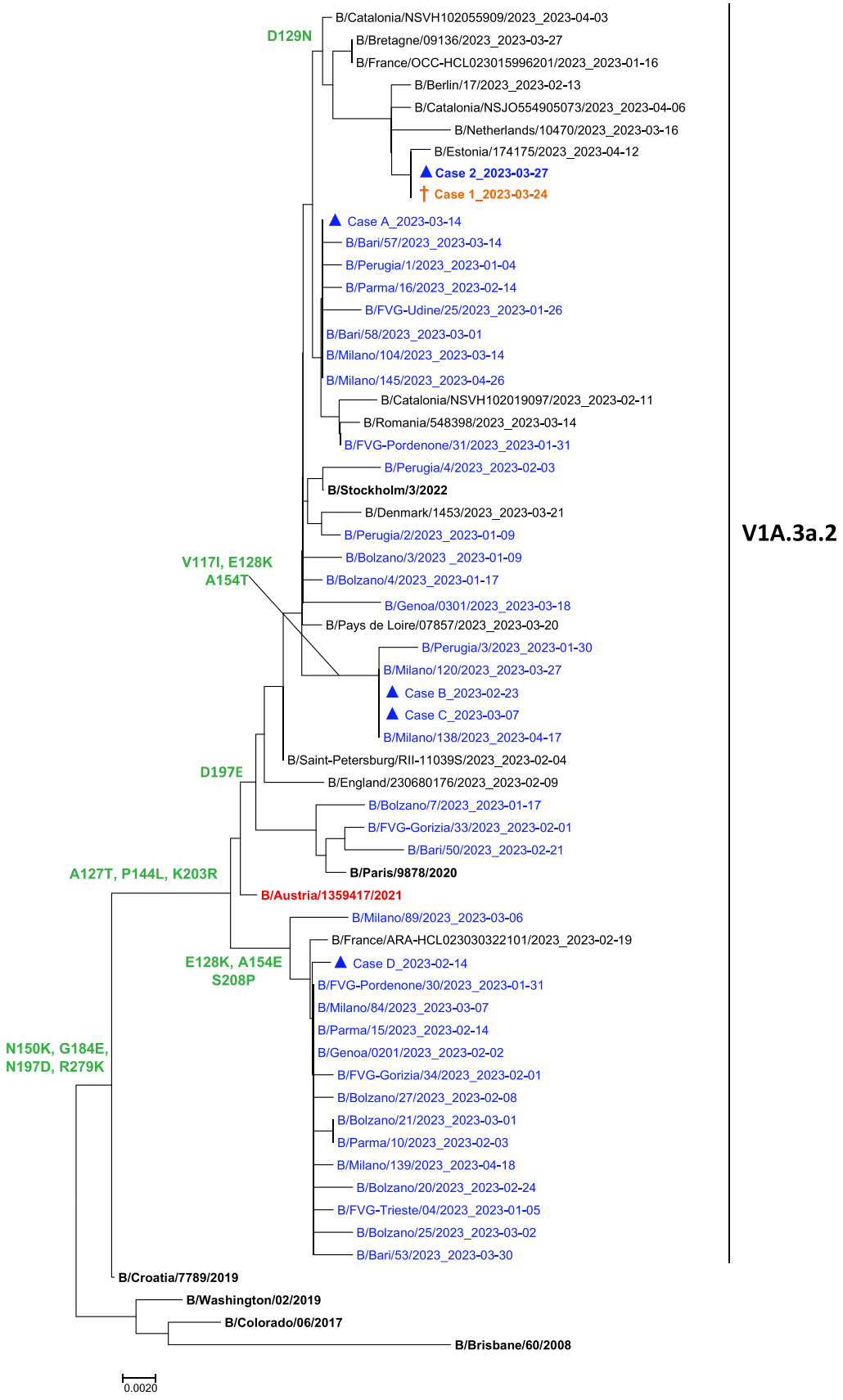

Fig. 1. Phylogenetic analysis of the HA1 nucleotide sequences of the influenza B/Victoria viruses from the fatal case (Case 1: in orange with †) and the family mild case (Case 2: in blue with ▲), compared to other B strains detected in Italy in the 2022–23 epidemic season (in blue) and to a selection of B/Victoria viruses detected in Europe in the same period (in black), retrieved from GISAID. In addition to the HA1 sequence from Case 1, further four sequences of influenza B/Victoria viruses from mild cases identified in children in the same Emilia-Romagna region (in blue with ▲) were included in the analysis and compared with the WHO-recommended vaccine strain for the Northern hemisphere (in red) and other reference strains (in black, bold font). Amino acid substitutions defining specific genetic clusters are indicated at nodes. The scale bar represents the nucleotide substitutions per site. The phylogenetic tree was constructed using the Neighbor-Joining method and the Kimura 2-parameter model, (MEGA software v.7.0.26).

Table 1
GISAID accession numbers of sequences from case 1 and case 2 and from other four mild cases identified in the same Region among children, Italy, 2023.

Sample	Isolate ID	HA segment ID	NA segment ID	PA segment ID
Case 1_2023-03-24	EPI_ISL_18059000	EPI2666703	EPI2665236	EPI2665237
Case 2_2023-03-27	EPI_ISL_18059131	EPI2666702	EPI2665403	EPI2665404
Case A_2023-03-14	EPI_ISL_18062864	EPI2666704	-	-
Case B_2023-02-23	EPI_ISL_18059069	EPI2666700	-	-
Case C_2023-03-07	EPI_ISL_18062766	EPI2666701	-	-
Case D_2023-02-14	EPI_ISL_18063187	EPI2666705	-	-

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Author contributions

PS conceived the study; SP and MF participated in the conception of the study and interpreted the data. SP, MF and SP (Sara Piacentini) wrote the first draft of the manuscript. GDM, ADM, LC and CF performed laboratory analyses at NIC. PA, MEC and LV collected samples and data and performed the laboratory analyses at regional level. GB, MC, GLC, RS and AR coordinated the collection of clinical and epidemiological data; ATP revised the manuscript; PS revised critically the manuscript and supervised. All authors participated in the analysis, interpretation of data and in the critical revision of the manuscript. All authors read and approved the final version of the manuscript.

References

[1] Tschöpe C, Ammirati E, Bozkurt B, Caforio ALP, Cooper LT, Felix SB, et al. Myocarditis and inflammatory cardiomyopathy: current evidence and future directions. Nat Rev Cardiol 2021;18(3):169–93. https://doi.org/10.1038/s41569-020-00435-x

[2] Saraiya N, Singh S, Corpuz M. Fatal influenza myocarditis with incessant ventricular tachycardia. BMJ Case Rep 2019;12:e228201. https://doi.org/10.1136/bcr-2018-228201

[3] Marchetti L, Gandolfo C, Maglioni E, Contorni M, Arena F, Cusi MG. Myocarditis requiring extracorporeal membrane oxygenation support following Influenza B infection: a case report and literature review. New Microbiol 2019;42:61–3.

[4] Puzelli S, Buonaguro FM, Facchini M, Palmieri A, Calzoletti L, De Marco MA, et al. Cardiac tamponade and heart failure due to myopericarditis as a presentation of infection with the pandemic H1N1 2009 influenza A virus. J Clin Microbiol 2010;48(6):2298–300. https://doi.org/10.1128/JCM.00418-10

[5] Puzelli S, Di Martino A, Facchini M, Fabiani C, Calzoletti L, Di Mario G, et al. Co-circulation of the two influenza B lineages during 13 consecutive influenza surveillance seasons in Italy, 2004-2017. BMC Infect Dis 2019;19(1):990. https://doi.org/10.1186/s12879-019-4621-z

[6] World Health Organization. Summary of neuraminidase (NA) amino acid substitutions associated with reduced inhibition by neuraminidase inhibitors (NAIs) (who.int).

[7] Puzelli S, Facchini M, Di Martino A, Fabiani C, Lackenby A, Zambon M, et al. Evaluation of the antiviral drug susceptibility of influenza viruses in Italy from 2004/05 to 2009/10 epidemics and from the recent 2009 pandemic. Antivir Res 2011;90(3):205–12. https://doi.org/10.1016/j.antiviral.2011.04.003

[8] World Health Organization. Summary of polymerase acidic (PA) protein amino acid substitutions analysed for their effects on baloxavir susceptibility (who.int).

[9] World Health Organization. Global Influenza Surveillance Network. Manual for the laboratory diagnosis and virological surveillance of influenza. ⟨https://www.who.int/publications/i/item/manual-for-the-laboratory-diagnosis-and-virological-surveillance-of-influenza⟩.

[10] Paddock CD, Liu L, Denison AM, Bartlett JH, Holman RC, DeLeon-Carnes L. Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza virus infection. J Infect Dis 2012;205:895–905.

[11] Grundtvig Skaarup K, Modin D, Nielsen L, Stæhr Jensen JU, Biering-Sørensen T. Influenza and cardiovascular disease pathophysiology: strings attached. Eur Heart J Suppl 2023;25(Suppl A):A5–11. https://doi.org/10.1093/eurheartjsupp/suac117

[12] European Centre for Disease Prevention and Control. Communicable disease threats report, 12–18 March 2023, week 11. ⟨https://www.ecdc.europa.eu/sites/default/files/documents/communicable-disease-threats-report-week-11-2023.pdf⟩.

[13] Han AX, De Jong SPG, Russel CA. Co-evolution of immunity and seasonal influenza viruses. Nat Rev Microbiol 2023;21:805–17.