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Short communication

Enterovirus D68-associated respiratory infection in southern Brazil, 2018 – A population-based laboratory surveillance

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A population-based laboratory surveillance protocol described by Nix and collaborators [11], and molecular epidemiology confirmed in 36/69 – 52.2% of enterovirus-positive samples collected through surveillance networks for severe acute respiratory infections (SARI) and influenza-like illness (ILI) in southern Brazil in 2018. This finding settles the sustained circulation of EV-D68 in southern Brazil.

Enterovirus D68 (EV-D68), a non-polio enterovirus (EV) was first isolated in 1962 in USA, and has since been reported sporadically in association with respiratory diseases [1]. In 2014, an outbreak of severe lower respiratory tract disease caused by EV-D68 occurred in North America; subsequently, it has been detected worldwide. An increase in EV-D68-associated acute flaccid paralysis (AFP) was observed in parallel during this period [2,3].

In Brazil, severe acute respiratory infections (SARI) are mostly not investigated for EV infections. However, we have been investigating human EV infections in SARI patients hospitalized at university hospital (HC/UFRP) since 2002 in Paraná (PR) state in southern Brazil, and approximately 10 % cases are EV-positive [4]. Although post 2015, the public health laboratory of Paraná State (LACEN-PR) made EV detection mandatory in samples collected from hospitalized and outpatients meeting the diagnostic criteria of SARI and influenza like-illness (ILI) via active surveillance, the EV-positive samples are not routinely genotyped [4]. Here, we report the results of EV-D68 investigation in respiratory samples of SARI and ILI patients from southern Brazil.

1. The study

From January 2018 to December 2018, a total of 11,922 samples obtained through SARI and ILI surveillance networks were evaluated for detection of 15 respiratory viruses. The surveillance system is based on the active investigation of outpatients who develop ILI and were attended in 21 sentinel community centers distributed in 17 municipalities with the largest population of the Paraná State and all hospitalized patients with SARI in private and public institutions of the State. The case definitions for influenza surveillance are as follows: (i) ILI – an acute respiratory infection with fever of ≥ 38 °C and cough, with onset within the last 10 days; (ii) SARI – presence of ILI and other indications of disease severity, such as oxygen saturation below 95 %, dyspnea, or respiratory discomfort.

From each SARI and ILI cases reported to the surveillance network within 5 days of symptom onset, combined oropharyngeal and nasopharyngeal swab samples were collected and sent to the public health laboratory to be analyzed. Samples were subjected to nucleic acid extraction with QIAsymphony DSP Virus/Pathogen Kit (Qiagen, Inc., Valencia, CA), according to the manufacturer’s instructions. One step qRT-PCR was performed by using the GoTaq® 1-Step RT-qPCR System (Promega, Madison, Wisconsin, EUA) in a final reaction volume of 10 μL using a 384 plate for detection on LightCycler®480II instrument (Roche, Indianapolis, IN). The respiratory viruses investigated were influenza A (IFA), influenza B, respiratory syncytial virus (RSV), rhinovirus (RV), enterovirus (EV), adenovirus, metapneumovirus, bocavirus, parainfluenza type 1, 2 and 3, coronavirus OC43, HKU1, NL63, and 229E [5–9]. Subsequently, all respiratory (SARI and ILI) samples positive for enterovirus (EV or EV/RV) were tested for EV-D68 using a specific real-time RT-PCR technique [10]. Ct values > 40.0 were considered negative. In addition, the test results were discarded for any sample whose internal control (human ribonuclease P gene) was negative.

Genetic material from a positive sample was amplified using the protocol described by Nix and collaborators [11], and molecular
Characterization of EV D-68 was carried out based on partial VP1 capsid gene region analysis. Nucleotide sequencing was analyzed using Enterovirus Automated Genotyping Tool [12].

Respiratory viruses were detected in 6535 (54.8 %) of SARI and ILI samples. The most frequently detected viruses were RV (15.1 %), IFA (11.7 %), and RSV (11.8 %). Enteroviruses (EV or EV/RV) were detected in 69 (1%) samples, being 39/69 (56.5 %) of SARI, and 30/69 (43.5 %) of ILI cases. Real-time RT-PCR EV-D68 assay was performed in all 69 EV-positive samples, and EV-D68 was detected in the 36 samples previously identified as EV/RV co-detection (Fig. 1). The phylogenetic and tool automated analysis of EV-D68 strain confirmed the specificity of the real time RT-PCR EV-D68 assay and suggested that the strain belongs to the B3 lineage (Fig. 2).

The monthly distribution of EV and EV-D68 detection is depicted at Fig. 3; most cases occurred in winter and summer seasons, and positive samples were found in 13 municipalities in the Paraná state. The median age of EV-D68-infected patients was 5 years (IQR, 1, 2–22, 7 y), 61 % were female, 61.1 % of cases had been notified as SARI, and 38.9 % as ILI in the surveillance report.

Previously, during the influenza pandemic in 2009, Carney et al. [13], reported EV-D68 detection in two Brazilian children who presented with respiratory illness in Salvador (Northeast Brazil). However, due to non-inclusion of EV-D68 in National Notifiable Diseases Surveillance System in the country, the public health burden of EV-D68 infection in Brazil remained underestimated.

After the 2014 outbreak in USA, EV-D68 has been identified in several regions at low frequency. However, the observation that it exhibits low viral circulation could be a consequence of an under-diagnosis, as most respiratory virus panels are not equipped to test for EV-D68.

Our findings are in agreement with earlier reports by Poelman et al. and Dyrdak et al, and showed that 50 % of the individuals who tested positive for EV-D68 in SARI and ILI surveillance were children, and many of them were hospitalized with SARI. In addition, we observed a high frequency of severe cases (> 60 %) and EV-D68 infected females. However, the disease severity of EV-D68 may have been overestimated, given that children not hospitalized owing to milder presentation were probably not sampled [14–17]. Further large-scale epidemiological information is needed to determine the actual burden of EV-D68. Moreover, contrary to a report from the USA [18], we observed higher frequency of EV-D68 infections in winter and summer. However, ongoing surveillance is needed to accurately define the seasonal pattern of this infection.

Being closely related, PCRs for EV and RV exhibit cross-reactions leading to underestimation of EV presence. Here, EV-D68 was found only in cases previously identified as EV/RV-co-detection. Thus, our data seem to support the presence of EV-D68 and human RV cross-reactivity, as reported by McAllister and collaborators [19]. Therefore, further tests more specific for EV-D68 detection were carried out to confirm its presence.

Phylogenetic studies based on the VP1 region of EV-D68 strains have shown the occurrence of clades A–D and subclades, with differences in the prevalence of the various subclades over time or in cocirculation [20]. There is no evidence that different EV lineages affect severity or clinical presentation. Acute flaccid paralysis (AFP) cases have been associated with EV-D68 detections from several lineages, including B1, B2 and B3. The B3 lineage is considered to have evolved recently, although it is not clear whether its epidemiological success is due to antigenic drift and whether its association with disease severity differs from that of other EV-D68 lineages such as B1, that was described in the 2014 outbreak [15,21].

In 2016, during an AFP outbreak in Argentina, some children tested positive for EV-D68, lineage B3, confirming the circulation of this virus in Latin America [22]. Therefore, considering this new evidence of EV-D68 circulation in the southern Brazil region, it is highly recommended that presence of EV-D68 infection be ruled out in all AFP patients, especially where it is preceded by respiratory symptoms.

This study presents the following limitations leading to EV-D68...
underestimation: (a) Although most EV-D68 detections are not associated with fever, samples selected were from influenza active surveillance, which were selected based on fever presence among other SARI or ILI symptoms. (b) EV-D68 was investigated only in EV-positive samples.

2. Conclusion

We found EV-D68 circulation across the Parana State in southern Brazil. Therefore, extensive population-based laboratory surveillance of EV-D68 will help evaluate the actual burden of this virus in the country and guide the implementation of effective measures to prevent future EV-D68 outbreaks.

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Fig. 2. Molecular Phylogenetic analysis of Enterovirus VP1 partial sequences. A: Analysis with reference sequences of human EV genotypes; B: Analysis with reference sequences of EV-D68 subclades. The evaluation was inferred under Maximum Likelihood method implemented in the MEGA7 software, based on the General Time Reversible substitution model, with 1000 replicates; reference strains were retrieved from the GenBank® database (http://www.ncbi.nlm.nih.gov/Genbank/) [the numbers shown to the left of the nodes represent bootstrap support values > 75. Bold indicates strain detected in this study (strain 2052-18, GenBank accession number MK628565). Scale bar indicates nucleotide substitutions per site].

Ethical approval

The Ethics Committee for Institutional Research of Hospital de Clínicas/UFPR revised and approved this study (#18714013.4.0000.0096).

All procedures followed in this study were in accordance with the 1964 Helsinki Declaration and later versions.

Informed consent

Written informed consent was obtained from patient’s parents when clinical data were collected.

CRediT authorship contribution statement

S.M. Raboni: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - review & editing.

H.I. Giamberardino: Investigation, Writing - original draft.

M.C. Fig. 3. Monthly occurrences of enterovirus positive samples for the year 2018, Southern Brazil. A: in relation to the number of ILI cases; B: in relation to the number of SARI cases.
Debure: Methodology, Validation, Writing - original draft. J.S. Santos: Methodology, Formal analysis, Writing - original draft.

Declaration of Competing Interest

None declared.

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