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Virological characterization of Parvovirus B19 isolated during the atypical 2023-2024 outbreak in France

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SUMMARY

Background: A Parvovirus B19 (B19V) outbreak has been reported in Europe in 2023–2024. The aims of this study were 1) to describe the incidence of primary cases from 2012 to 2024 in one French hospital 2) to analyze the genome of 2023 strains 3) to identify virological profiles according to the clinical presentations of B19V infection.

Methods: The incidence of B19V primary cases was studied through an interrupted time-series analysis. Genomes of 2023 strains were sequenced in the NS1-VP1u region. Blood viral loads, IgG and IgM levels were analyzed in 158 cases according to clinical manifestations with Kruskal-Wallis test and a machine learning approach based on k-nearest neighbors.

Results: During the 2023–2024 B19V outbreak, there was an 8-time increase in the incidence of B19V infections compared with pre-pandemic levels (8.25 (95%CI: 5.79–11.76)). The 2023 strains belonged to genotype 1a and were closely related to pre-2019 strains. Blood viral loads were significantly different between clinical presentations ($p < 0.0001$). Machine learning allowed us to classify 68.8% (95% CI: 60.9–75.9) patients into the correct clinical group.

Conclusions: The 2023–24 epidemic is probably due to the reemergence of the pre-2019 strain. The virological profiles highlighted in this study could assist in accurately interpreting virology results.

Introduction

In Europe, human parvovirus B19 (B19V) is endemic with annual epidemics of varying size occurring in spring and early summer. Larger epidemics occur every 4 to 5 years. In July 2023, we reported an unusually large B19V outbreak starting in April 2023.[1] Since then, a similar high circulation of B19V has been reported in 2024 in different European countries such as Denmark,[2] the Netherlands,[3] and Israel.[4] However, very little data are yet available on the molecular epidemiology of the B19V strains responsible for this large epidemic. In France, there is no national surveillance program for B19V infections. As a proxy, we monitored new primary B19V infection cases diagnosed in our hospital from May 2023 onwards. Moreover, a sample of strains circulating in 2023 was genotyped by sequencing the NS1-VP1u region. Molecular sequences were compared to those of pre-COVID-19 circulating strains reported in GenBank.

Diagnosis of B19V-related clinical syndromes (*erythema infectiosum*, acute transient red cell aplasia, chronic anemia, myocarditis or other post-viral complication, hydrops fetalis) is mainly based on the combined determination of B19V DNA load by PCR in blood and specific IgG and IgM detection.[5] In the study from Gallinela et al., positive B19V IgM and positive B19V PCR in blood were detected in 60% and 79% of B19V-related cases, respectively; the combined positivity of PCR and/or serology allowed the higher sensitivity. However, comprehensive patterns of those two B19V virological markers (PCR and serology) according to the type of clinical presentation have been rarely described in the literature. In the current study, we retrospectively identified B19V-related clinical

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cases through a search of our center's laboratory database and of clinical files from January 2012 to May 2024. This allowed the description of 4 virological profiles specific of 4 clinical presentations of B19V infection (*erythema infectiosum*, acute transient red cell aplasia, post-viral complication, and chronic anemia in immunocompromised patients).

Materials and methods

Study design and setting

We identified cases of B19V infections using patient-level electronic health records of Necker-Enfants malades Hospital, Paris, France, from January 2012 to May 2024. Necker-Enfants malades is a 600-bed tertiary care hospital, including a referral center for sickle cell disease, transplant units for adults and children, a pediatric cardiology unit and a maternity. Therefore, in this hospital, the full spectrum of B19V infections is represented. Thus, B19V infection data from our center are an indirect reflection of the burden of B19V epidemics and can be used to analyze the trend of the epidemics in France in the absence of organized surveillance. For primay infection incidence data, we selected all patients (regardless of age) with a B19V primary infection, defined by the association of a positive B19V PCR and positive B19V IgM.

Genotyping of B19V strains was performed by sequencing samples from a subselection of samples including patients with severe cases (patients requiring admission to the intensive care unit) and non-severe cases (not hospitalized or hospitalized in a general medical ward) and including cases representative of the 4 different clinical presentations.

For the analysis of virological profiles, all patients with a positive B19V PCR in blood and a result of B19V serology from the same day and clinical symptoms related to B19V infection according to the clinician and as reported in the electronic health record were selected. Patients were classified into 4 groups according to their clinical presentation (group 1= immunocompetent patients with *erythema infectiosum*, group 2= acute transient red cell aplasia in patient with chronic hemolytic anemia, group 3= myocarditis and post-viral complication, group 4= chronic anemia in immunocompromised patients). As a control group (group 5), we included patients with a positive B19V PCR in blood and a result of B19V serology and clinical symptoms that were not considered related to B19V infection as reported by the clinician in the health record.

Laboratory analysis

Extraction of DNA. DNA was extracted from 200 µl of EDTA blood by the easyMAG™ instrument (Biomérieux, France) and eluted in 50 µl of buffer according to the manufacturer's instructions.

B19V quantitative polymerase chain reaction (qPCR) was performed with the B19-R Gene Argene kit (Biomérieux, France). Results were reported in log IU/mL. The lower limit of detection (LOD) was 280 IU/mL (2.4 $\log_{10}$ IU/mL). The lower limit of quantification (LOQ) was 700 IU/mL (2.8 $\log_{10}$ IU/mL). Positive viral loads less than the LOQ were reported as half the threshold value (2.5 $\log_{10}$ IU/mL).

B19V IgG and IgM assays were performed with the Liaison Biotrin B19V kit on the Liaison XL platform (Diasorin, France). Results were reported in arbitrary units (U/mL). The manufacturer considers a result < 0.9 U/mL as negative, a result > 1.1 U/mL as positive, and a result between 0.9 – 1.1 U/mL as equivocal. As routine practice, in cases with positive anti-B19 IgM antibodies, the serum is tested for the presence of B19 DNA by PCR in order to confirm or not a primary B19 infection.

Other serology tests: The standard etiological work-up for myocarditis includes a panel of serology including EBV, CMV and parvovirus B19. In cases where IgM antibodies against CMV or EBV were positive, a PCR assay was performed to confirm or exclude the presence of viral DNA and to overcome IgM cross-reactivity.

B19V Sanger genotyping was based on sequencing 724 base pairs (bp) of the NS1-VP1u region as already described.[6] Briefly, a nested PCR was performed on extracted DNA using the following primers for the first PCR: forward primer 5'-AGCAGTGGTGGTGAAAGCT-3' and reverse primer 5'-CCCAGGCTTGTGTAAGTCT-3'; and the following primers for the nested PCR: forward primer 5'-AGCAGTGGT GGTGAAAGCTCTGAA-3' and reverse primer 5'-CCAGGCTTGTGTAAG TCTTCACTAGA-3'. Amplifications were carried out using the Eppendorf Mastercycler (Eppendorf, Germany). The PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide. Products for sequencing were purified with the QIAquick PCR Purification kit (Qiagen, Germany). Sequencing was performed with the nested PCR primers using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). Sequences were edited and aligned with references in BioEdit v7.1 software and subjected to NCBI BLAST analysis.

Phylogenetic analysis. Phylogenetic trees were performed using Phylogeny.fr in the " one click mode " using default settings (https://www.phylogeny.fr/).[7] Succinctly, the alignment was done with MUSCLE 3.8.31, alignment refinement was performed with Gblocks 0.91b, phylogeny was done using PhyML 3.1/3.0 aLRT,[8] which is based on the maximum-likelihood principle, and the phylogenetic tree was prepared using TreeDyn 198.3.[9]

Statistical analysis

Descriptive statistics were used to characterize the data and study population. Specifically, medians and interquartile ranges were calculated for continuous variables, and proportions were determined for qualitative variables using GraphPad Prism 8.0 (GraphPad Prism Software Inc.).

For the epidemiologic part of the study, monthly incidence (i.e., number of cases) of primary B19V infections was analyzed using an interrupted time-series analysis relying on Poisson regression modeling with seasonal adjustment. Initially, we checked that the data did not exhibit overdispersion. To address seasonality, we included Fourier terms in the model (i.e., a pair of sine and cosine terms). Focusing on two significant events, the first national COVID-19 lockdown in March 2020 and the unusually high levels of B19V infections in March 2023, we divided the data into three distinct periods for analysis: January 2012 to March 2020 (pre-pandemic period, 99 time points), April 2020 to March 2023 (pandemic period, 36 time points), and April 2023 to May 2024 (post-pandemic period, 14 time points). We then calculated incidence rate ratios to assess temporal associations between these periods and B19V incidence. Values < 1 indicate a decrease in incidence, while values > 1 indicate an increase. All analyses were performed using Stata/SE v18 (StataCorp, College Station, Texas).

The Kruskal-Wallis test was used to compare each biomarker between the 5 groups. Wilcoxon-Mann-Whitney test with Bonferroni correction was used to compare each group pairwise for each biomarker. GraphPad Prism 8.0 (GraphPad Prism Software Inc.) and R Studio 4.0.0 (R Studio software Inc.) were used for these analyses. Then, a machine learning approach using k-nearest neighbors (with k=5) discriminant analysis was employed to classify patients into clinical groups based on their virological profiles (viral load, IgG and IgM). The accuracy of the machine algorithm was evaluated using leave-one-out predictions. This analysis was performed using Stata/SE v18 (StataCorp, College Station). Statistical significance was defined by a p-value < 0.05. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) with a 95% confidence interval (95%IC) were

calculated for each of the studied groups using R Studio 4.0.0 (R Studio software Inc.).

Ethics

This study relied on existing data collected as part of usual care in a single center. As per French regulation, such retrospective analysis of pre-existing anonymized data does not require formal approval by the Institutional Review Board. In our hospital, all patients receive written generic information that the data collected during routine care may be used for research purposes, and they are offered the right to withdraw at any time. The study was submitted to our institution's data protection office, which deemed the study to conform to the General Data Protection Regulation (registration number 2023–0802181444).

Results

Epidemiologic trends

A total of 168 primary B19V infections occurred over the study period. Epidemics from 2012 to 2019 showed similar trends, usually with between 0 and 3 incident cases per month, except in 2013 and 2018, when the epidemics were slightly larger. This was followed by a 3-year period (2020–2022) with an extremely low incidence of primary cases. In 2023 and 2024, the epidemics were abnormally high, peaking in July 2023 (11 cases) and in May 2024 (9 cases). This post-COVID-19 epidemic corresponded to an 8-time increase in the incidence of B19V infections compared with the pre-pandemic period (incidence rate ratio (8.25 [95%CI: 5.79–11.76]) (Fig. 1).

In total, 95 new primary B19V cases were diagnosed between January 2023 and May 2024, comprising 20 patients with *erythema infectiosum* (among whom 5 pregnant women with severe fetal infection), 50 patients with acute red cell aplasia (43 with sickle cell disease, 5 with hereditary spherocytosis, 1 with G6PD deficiency and 1 with both sickle cell disease and G6PD deficiency), 8 patients with myocarditis, 4 patients with nephrotic syndrome, 1 patient with Guillain-Barré syndrome, 1 patient with Kawasaki disease and 11 immunocompromised patients who developed anemia (6 with primary immunodeficiency, 4 kidney transplanted recipients and 1 liver transplanted recipients).

Genotyping of 2023 strains

Sequencing was achieved in 27 samples chosen from cases with various clinical presentations: 2 *erythema infectiosum,* 17 acute red cell aplasia (14 sickle cell disease and 3 hereditary spherocytosis), 3 severe myocarditis, 1 Guillain-Barré syndrome, 3 anemia in immunocompromised patients and 1 intrauterine fetal death. Five of these cases were severe, leading to death (3 myocarditis) or needing intensive care unit admission (1 Guillain-Barré syndrome and 1 with sickle cell disease patient).

The 27 sequences were highly homogeneous, and all belonged to the 1a genotype (Fig. 2. A). The intra-sample nucleotides variability of these 27 strains ranged from 0.14% to 1.93%. These 2023 strains were very close to the strains that circulated in France during the decades preceding the COVID-19 pandemic, particularly in 2019 (Fig. 2. B). The nucleotide variability between samples from 2023 and those from 2019 (pre-COVID-19 period) ranged from 0.14% to 2.35%. The 27 sequences are available in GenBank with accession numbers (**PP951097**, **PP951098**, **PP951099**, **PP951100**, **PP951101**, **PP951102**, **PP951103**, **PP951104**, **PP951105**, **PP951106**, **PP951107**, **PP951108**, **PP951109**, **PP951110**, **PP951111**, **PP951112**, **PP951113,** **PP951114**, **PP951115**, **PP951116**, **PP951117**, **PP951118**, **PP951119**, **PP951120**, **PP968059, PP968060** and **PP968061**).

Virological profile according to B19V-related clinical manifestations

Among the 168 primary B19V infection over the study period (2012–2024), 140 patients were selected because they had a positive B19V PCR in blood, a B19V serology result on the same day, and an available health report reporting that clinical manifestations were considered as related to B19V infection by the clinician in charge.

18 patients had low equivocal levels of IgG (between 0.9 and 5 U/mL). However, these 18 patients had a confirmed positive B19V PCR and 13 had strongly positive IgM. These concurrent IgM and PCR results allow us to confirm that in these cases, although the IgG were low, there were true positive.

In all cases of B19V myocarditis with positive anti-B19V IgM, EBV and CMV IgM were negative except for one patient. This patient had a negative CMV PCR and a positive B19V PCR, confirming the specificity of anti-B19V IgM.

The patient's characteristics are detailed in Table 1.

The patients were classified into 5 groups as described above (Table 1) and virological data were analyzed according to this classification (Figs. 3A, 3B and 3C). For the 24 immunocompetent patients (group 1), blood viral loads were moderate (median = 5.78 $\log_{10}$ IU/mL), IgM levels were high (median = 39 U/mL), and IgG levels were moderate (median = 19 U/mL). For the 68 patients with acute red cell aplasia (group 2), blood viral loads were extremely high (median = 8.25 $\log_{10}$ IU/mL), IgM levels were high (median = 48 U/mL), and IgG levels were low (median = 8.61 U/mL). For the 25 patients with post-viral complication (group 3), blood viral loads were moderate (median = 4.89 $\log_{10}$ IU/mL), and the serology showed negative IgM in 60% of cases (median = 1.90 U/mL) and high IgG levels (median = 33.20 U/mL). For the 23 immunocompromised patients with anemia (group 4), blood viral loads were high (median

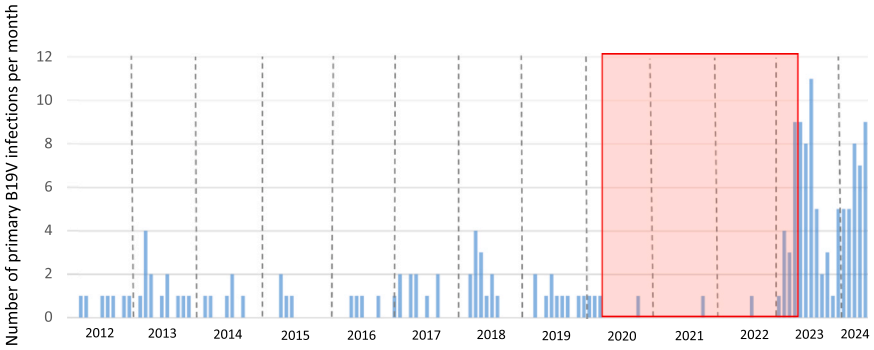

Fig. 1. Number of primary B19V infections per month from January 2012 to May 2024. The red square indicates the COVID-19 pandemic in France (April 2020 – April 2023). The vertical dotted lines demarcate each year. Poisson modeling with seasonal adjustment identified a decrease in B19V primary infections during the COVID-19 pandemic (incidence rate ratio 0.48 [95%IC 0.24–0.95]) and an outbreak starting in April 2023 (incidence rate ratio 8.25 [95%IC 5.79–11.76]).

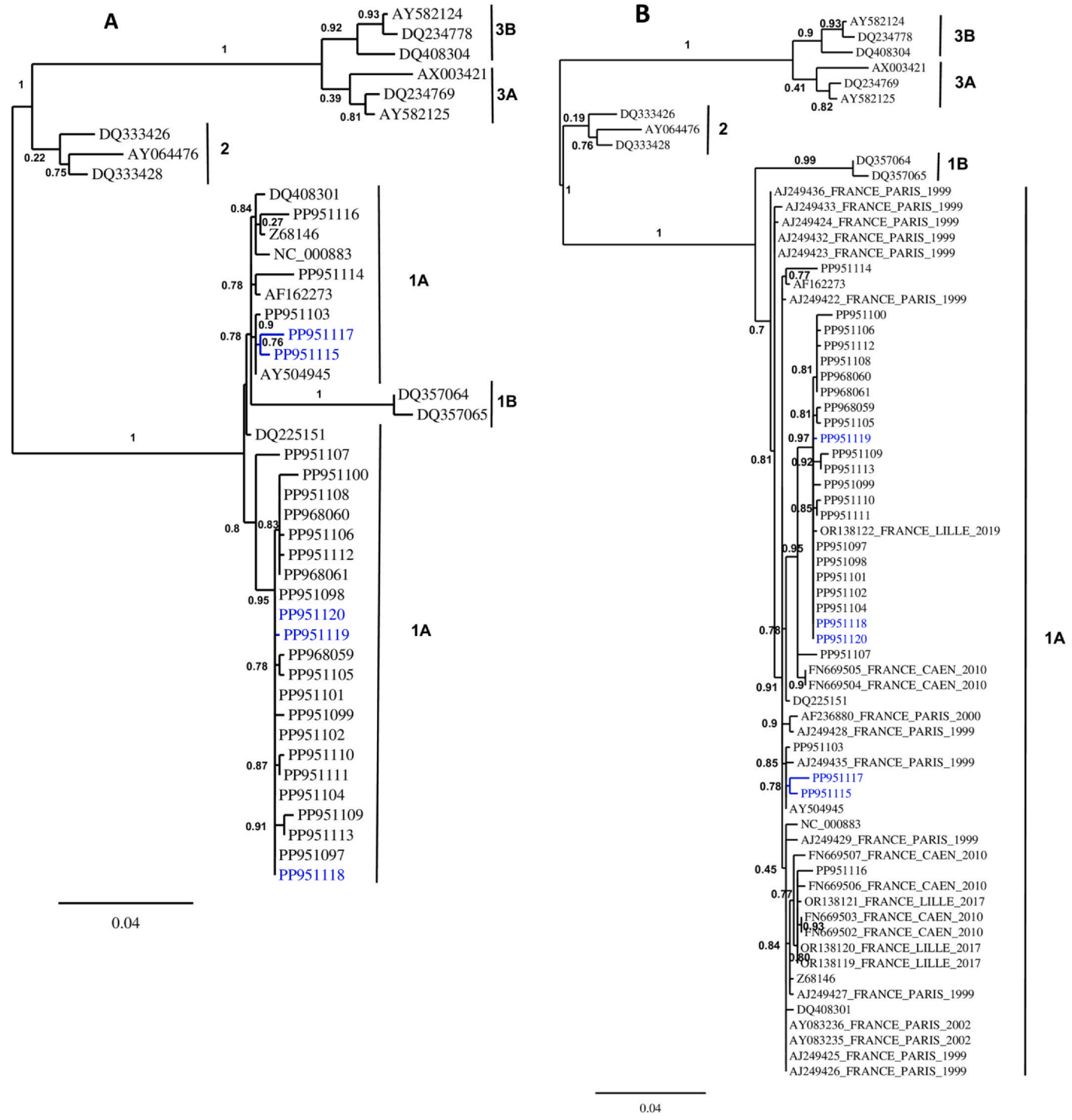

Fig. 2. A and 2.B. Molecular analysis of B19V from the 2023 epidemic (n=27). B19V Sanger genotyping was based on sequencing 724 base pairs of the NS1-VP1u region. The 27 sequences of Necker sample are available in GenBank with accession numbers (PP951097 – PP951120 and PP968059 – PP968061). Sample numbers in blue correspond to patients with a severe clinical form of B19V. 3.A. Phylogenetic tree of the twenty-seven B19V sequences from Necker Hospital compared to reference sequences of the different B19V genotypes (1a/b, 2 and 3a/b) available in GenBank. 3.B. Phylogenetic tree of the twenty-seven B19V sequences from Necker Hospital compared to genotype 1a B19V sequences found in France in the years preceding the COVID-19 pandemic. These are indicated by the city and year of their discovery. Bootstrap values are shown in the trees.

= 7.70 $\log_{10}$ IU/mL), IgM levels were low (median = 10 U/mL) as were IgG levels (median = 10 U/mL). Finally, for the 18 patients of the control group, blood viral loads were very low, with 56% of patients having values below the LOQ (median = 2.54 $\log_{10}$ IU/mL), IgM were negative (median = 0.32 U/mL) and IgG levels were moderate (median = 17 U/mL).

For each biomarker (blood viral load, IgG, and IgM), a significant difference between at least two of the studied groups was evidenced using the Kruskal-Wallis test ($p < 0.0001$). Comparison of each group pairwise and for each biomarker using the Wilcoxon-Mann-Whitney test with Bonferroni correction evidenced a significant difference between all groups except: 1) between groups 2 and 4 (p=0.470) (Fig. 3A) for blood viral load; 2) between groups 1 and 3 (p=0.194), 2 and 4 (p=0.315) and 1 and 5 (p=0.731) for IgG (Fig. 3B); 3) and between groups 1 and 2 (p=0.546), 3 and 4 (p=0.139), 3 and 5 (p=0.488) and 4 and 5 (p=0.054) for IgM (Fig. 3C).

Table 1
The patient characteristics.

Patients with parvovirus B19-related symptoms from 2012 to 2024					
	Total	Immunocompetent patients with *Erythema infectiosum*	Acute red cell aplasia in patients with chronic hemolytic anemia	Myocarditis and other post-viral complications	Anemia in immunocompromised patients
Clinical and biological characteristics					
Number of cases	140	24	68	25	23
male/female	60/80	5/19	34/34	10/15	11/12
Age, years (median, (IQR))	8.9 (5.2 - 14.4)	12.5 (9.3 - 34.1)	8.5 (5.2 - 12.5)	2.1 (0.9 - 7.8)	14.2 (6.8 - 43.4)
< 18 years old/ > 18 years old	115/25	14/10	65/3	22/3	14/9
Hemoglobin level (g/dL)[a,b] (median, (IQR))	7.0 (5.3 - 9.4)	11.6 (10.9 - 12.6)	5.5 (4.4 - 7.0)	9.0 (7.3 −11.2)	7.1 (6.1 - 8.1)
Reticulocytes count (G/L)[a,b] (median, (IQR))	20.5 (5.9 - 54.0)	37.3 (7.7 - 110.5)	21.1 (8.5 - 34.4)	73.7 (27.9 - 89.5)	4.4 (2.4 - 7.3)
CRP level (mg/L)[a,b] (median, (IQR))	9.4 (5.1 - 54.4)	3.5 (1.3 - 9.7)	10.2 (6.0 - 41.5)	16.5 (6.3 - 73.5)	34.9 (3.2 - 150.3)
Ferritin (µg/L)[a,b] (median, (IQR))	562.0 (97.5 - 4184.0)	52.0 (22.0 - 151.0)	734.0 (274.0 −5706.0)	175.5 (62.0 −988.5)	1928.0 (344.0 - 6274.0)
Pregnant women, n (%)	8 (5.7%)	8 (33.3%)	0	0	0
Sickle cell disease (SCD), n (%)	60 (42.9%)	0	60 (88.2%)	0	0
Spherocytosis, n (%)	7 (5.0%)	0	7 (29.2%)	0	0
Glucose-6-phosphate dehydrogenase (G6PD) deficiency, n(%)[b]	4 (2.9%)	0	4 (16.7%)	0	0
Myocarditis, n (%)	19 (13.6%)	0	0	19 (76.0%)	0
Nephrotic syndrome, n (%)	4 (2.9%)	0	0	4 (16.0%)	0
Guillain-Barre syndrome, n (%)	1 (0.7%)	0	0	1 (4.0%)	0
Kawasaki disease, n (%)	1 (0.7%)	0	0	1 (4.0%)	0
Primary immune deficiency, n (%)	7 (5.0%)	0	0	0	7 (29.2%)
Kidney transplant, n (%)	8 (5.7%)	0	0	0	8 (32.0%)
Liver transplant, n (%)	2 (1.4%)	0	0	0	2 (8.0%)
Heart transplant, n (%)	2 (1.4%)	0	0	0	2 (8.0%)
hematopoietic stem cell transplant, n (%)	1 (0.7%)	0	0	0	1 (4.4%)
Malignant hémopathy, n(%)	3 (2.1%)	0	0	0	3 (13.0%)
Other immunosuppressive situations, n (%)	2 (1.4%)	0	0	0	2 (8.0%)
Immunosuppressive therapy, n (%)	14 (10.0%)	0	0	0	14 (56.0%)
Treatments used					
Red cell transfusion, n (%)	93 (66.4%)	3 (12.5%)	60 (88.2%)	10 (40.0%)	19 (82.6%)
Intravenous immunoglobulin administration, n (%)	18 (12.9%)	0	1 (1.5%)	7 (28.0%)	10 (43.5%)
Corticoid therapy, n (%)	19 (13.6%)	0	2 (2.9%)	16 (64.0%)	1 (4.4%)
Pain requiring morphine IV administration in SCD patients, n (%)	33 (23.6%)	0	27 (39.7%)	4 (16%)	2 (8.7%)
Prognosis					
Hospital admission, n (%)	117 (71.4%)	5 (20.8%)	66 (97.1%)	25 (100.0%)	21 (91.3%)
Admission to the intensive care unit, n (%)	22 (15.7%)	0	3 (4.4%)	16 (64.0%)	3 (13.0%)
Fatal outcome, n (%)	4 (2.9%)	0	0	4 (16.0%)	0

[3]among them, 3 have sickle cell disease and G6PD deficiency.

[a] At the time of diagnosis of primary infection.

[b] Biological data from all patients in the cohort (n=140), Immunocompetent patients with Erythema infectiosum (n=24), Acute red cell aplasia in patients with chronic hemolytic anemia (n=68), Myocarditis and other post-viral complications (n=25) and Anemia in immunocompromised patients (n=23) were available for hemoglobin level in 133, 18, 68, 25 and 22 patients, reticulocytes count in 112, 6, 68, 19 and 19 patients, CRP level in 122, 14, 65, 23 and 20 patients, Ferritin in 45, 7, 19, 8 and 11 patients respectively.

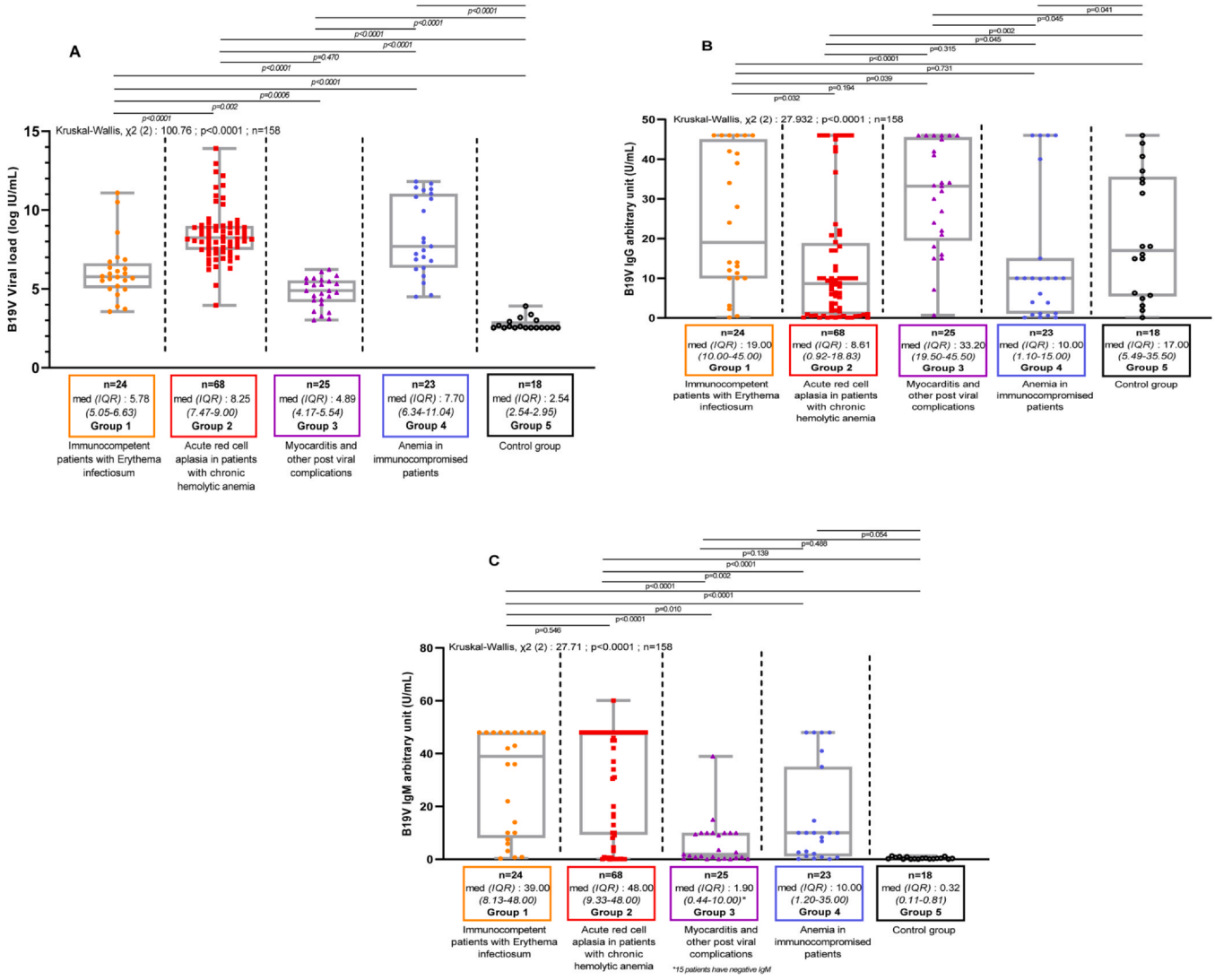

Fig. 3. B19V virological markers according to clinical presentation. According to the viral load (log IU/mL) and serology (IgG and IgM in arbitrary unit: U/mL), we identify 4 different virological patterns typical of 4 different clinical situations. As a control group (group 5), we included patients with a positive B19V PCR in blood and a result of B19V serology and clinical symptoms that were not considered related to B19V infection as reported by the clinician in the electronic health record. The boxes represent interquartile ranges with the horizontal line indicating the median value and the whiskers showing minimal and maximal value. A Kruskal-Wallis test was conducted for each biomarker. The *p* value (two-tailed) was calculated using Wilcoxon-Mann-Whitney test with Bonferroni correction. ***A.*** Blood B19V load according to the clinical presentation. ***B.*** IgG anti-B19V according to the clinical presentation. ***C.*** IgM anti-B19V according to the clinical presentation.

The machine learning approach allowed the classification of 68.8% of the patients into the correct group (accuracy = 68.8% (95% CI: 60.9–75.9)) (Table 2). In more than two-thirds of cases, the 3 biomarkers allowed distinguishing between the different clinical syndromes and correctly classifying patients. Upon closer examination of the results, classification errors were predominantly observed for groups 1 and 4, with a low sensitivity of 41.7% (95%CI: 22.1–63.4) and 22.7% (95%CI: 7.8–45.4) respectively. By contrast, the classification in group 3 and group 5 were highly accurate, with a sensitivity of 72.0% (95%CI: 50.6–87.9) and 94.4% (95%CI: 72.7–99.9) and a specificity of 90.9% (95%CI: 84.7–95.2) and 96.4% (95%CI: 91.8–98.8), respectively (Supplementary Figure 1).

Discussion

The B19V outbreak described in 2023 is still ongoing in 2024. This is unusual since B19V is endemic in France, with typically small outbreaks every 4–5 years in the late winter and early spring, as demonstrated here by the trends of the epidemics from 2012 to 2019 in our center. The current 2023–2024 outbreak is unusually prolonged and also displays an atypical seasonal pattern.[10,11]

All 2023 strains sequenced in this study belonged to genotype 1a with very low inter-strains distance (maximum 1.93% nucleotide difference). Moreover, 2023 strains were closely related to the strains circulating in the pre-COVID-19 period (less than 2.35% nucleotide difference). Sequencing was done in the NS1-VP1u region, which comprises the VP1u sequence known to encompass a dominant immune region containing the major neutralizing epitopes.[12] Therefore, this method allows classification in known genotypes (1,2 or 3) and genotypes subtypes (1 A, 1B, 3 A and 3B) and also allows the identification of new genotypes or subtypes.[6,13] Genotype 1 is predominant in most of the world, notably in Europe.[14] Genotype 2 is now found sporadically[15] but was predominant before the years 1950–1960 when it was replaced by genotype 1.[16] Genotype 3 has a low circulation and is found in specific geographic areas, notably in Africa.[14] The low inter-strains nucleotide difference described in this study is consistent with the results from studies based on whole-genome sequencing (either by the Sanger technique or by next-generation sequencing (NGS)) that reported a genetic distance

Table 2
Machine learning results based on a K-nearest Neighbors (k=5) algorithm.

	Observed					
Leave-one-out prediction	immunocompetent patients with *erythema infectiosum* (Group 1)	Acute red cell aplasia in patients with chronic hémolytic anemia (Group 2)	Myocarditis and other post viral complications (Group 3)	Anemia in immunocompromised patients (Group 4)	Control group (Group 5)	Total
immunocompetent patients with *erythema infectiosum* (Group 1)	**10**	**1**	**2**	**1**	**1**	**15**
Acute red cell aplasia in patients with chronic hémolytic anemia (Group 2)	**6**	**58**	**1**	**12**	**0**	**77**
Myocarditis and other post viral complications (Group 3)	**6**	**2**	**18**	**4**	**0**	**30**
Anemia in immunocompromised patients (Group 4)	**1**	**7**	**0**	**5**	**0**	**13**
Control group (Group 5)	**1**	**0**	**4**	**0**	**17**	**22**
Total	**24**	**68**	**25**	**22**	**18**	**157**[a]

Accuracy = (10+58+18+5+17)/157 = 108/157=68.8% (95%CI: 60.9-75.9).
Classification of patients (n=158) into each clinical presentation group based on three B19V biomarkers (viral load in blood, IgG, and IgM) using a machine learning approach with the K-Nearest Neighbors algorithm (k=5). One patient was excluded from the analysis due to being classified as an tie by the K-Nearest Neighbors algorithm.
[a] A patient could not be classified into a group by the K-Nearest Neighbors algorithm and was therefore excluded from the machine learning analysis as an tie.

between the three genotypes of around 10% but a low intragenotype distance (1–3%)[5] with diversity values in the range of 0.062–0.079 reported for genotype 1.[17]

Since no new NS1-VP1u genotype or subtype was identified in the 2023 strains, it is unlikely that the magnitude of the current epidemic is linked to the emergence of a new strain variant. The persistent outbreak is more likely due to a reduction in herd immunity in relation to the low levels of B19V circulation over the 3 first years of the COVID-19 pandemic. Indeed, reports from blood transfusion facilities showed that the annual prevalence of B19V DNA positive pools in the period 2015–2024 were the lowest during the years 2020, 2021 and 2022, with a high rebound in 2023 and 2024.[18,19] B19V is spread through respiratory droplets, and the low circulation of B19V is probably related to the measures implemented to limit the spread of SARS-CoV-2. It was also reported that these measures reduced the transmission of respiratory viruses such as respiratory syncytial virus during the COVID-19 period, followed by an unusual delayed outbreak,[20] as well as other respiratory tract pathogens such as *Bordetella pertussis*.[21] The clinicians at our hospital had the impression that there were more severe cases linked to B19V (myocarditis in particular) and wondered whether a particular strain could explain these cases. However, the nucleotide sequences from severe cases were similar to those of non-severe ones. This is consistent with the literature, which shows that the clinical spectrum of B19V infection is identical, regardless of the genotype or subtype. The higher-than-usual number of severe cases is, therefore, probably only related to the scale of the epidemic.

The different virological profiles according to clinical manifestations reported here are in accordance with the known pathophysiology and temporal sequence of infection for each clinical syndrome.[22] Around a week after the contamination, the B19V virus circulates in the bloodstream and hits its primary target, the erythroblasts. Replication in erythroblasts is asymptomatic in immunocompetent patients but can lead to acute red cell aplasia in individuals with chronic hemolytic anemia, explaining the typical virological profile found in these cases with extremely high blood viral load corresponding to its peak, high levels of IgM and low or yet undetectable IgG level. *Erythema infectiosum* appears around two weeks after the contamination at the same time as specific antibodies arise, explaining the typical virological profile associating high IgG and IgM levels and a lower viral load than in the previous group since blood DNAemia has already decreased at that stage. Myocarditis and nephrotic syndrome are post-viral complications presenting with extensive mononuclear tissue infiltrates[23] and no detection of viral protein, pleading for a major role of post-infectious inflammation in their etiology.[24,25] The typical virological profile reported here in these complications combines highly positive IgG with low or already negative IgM and blood viral load mostly between 4 and 5 $\log_{10}$ IU/mL.

In the control group with clinical symptoms not related to B19V, the blood viral load was very low, and IgG levels were positive with negative IgM. Such low viral loads (median 2.7 and 2.2 $\log_{10}$ IU/mL) have been reported in 0.1 to 0.9% of asymptomatic blood donors and 2.6 to 5% of asymptomatic bone marrow or kidney transplanted recipients with evidence of B19V past infection (positive IgG and negative IgM).[26–28] In these cases, as in those from the control group of this study, low positive B19V blood viral load suggests persistent infection or reactivation.

In clinical practice, a low or intermediate B19 blood viral load might be difficult to interpret, and its causality to explain the clinical symptoms might be difficult to ascertain. In this study, we were able to define 5 statistically different virological profiles based on viral load, IgG, and IgM, specific of 5 different clinical syndromes. It is particularly relevant to correctly identify patients with B19V post-infection syndromes such as B19V-related myocarditis, or immunosuppressed patients with anemia, from patients with

persistent infection or reactivation. Indeed, patients with B19V-related myocarditis can benefit from corticosteroid therapy or other immunosuppressive therapy such as interferon beta-1b,[29] and immunosuppressed patients with B19V-related anemia can benefit from high-dosage intravenous immunoglobulin infusion.[30] On the other hand, a wrong attribution of symptoms to B19V infection may be responsible for overlooking alternative etiologies. Our machine learning results based on virological data demonstrated high sensitivity and specificity in identifying patients with myocarditis and post-viral complications and those with only persistent infection or reactivation. The virological profiles highlighted in this study could assist virologists in accurately interpreting virology results.

Strengths and limitations

The main strength of this study is that it relies on data from a tertiary care institution where recruitment has remained homogeneous over an extended timeframe and where the techniques for detecting B19V infection (PCR and serology) have not changed throughout the study period. A weakness of the study is that the complete genome sequence was not studied by NGS. Indeed, by sequencing only the NS1/VP1u part of the genome, we could have missed the emergence of a new variant. However, this seems unlikely given that all the genotypes and subtypes currently described for this virus diverge on the dominant epitopes included in this sequence. Another potential weakness of the study is that a single kit was used to measure B19V IgM with possible cross-reactivity. However, to overcome this problem B19 PCR was systematically performed in positive IgM sera to confirm primary B19 infection.

Conclusion

Our study shows a persistent and unusual outbreak of B19V infections, and levels of suspicion should remain high among frontline physicians. We found no evidence of the emergence of a new strain that could explain the current B19V epidemic in France, and the 2023 strains were very close to those that circulated in France during the decades preceding the COVID-19 pandemic. In this current context of a large and prolonged B19V outbreak, the description of the various virological profiles may help virologists and clinicians better interpret B19V viral profiles. Confirming or ruling out a recent B19V infection is essential to guide appropriate patient management.

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Author contributions

Dr Marianne Leruez-Ville designed the study, coordinated and supervised data collection, attest to the data and analysis and drafted the initial manuscript. Dr Nicolas Veyrenche analyzed the data, performed the statistical analysis, and drafted the initial manuscript. Prof. Jéremie F. Cohen performed the statistical analysis, critically reviewed, and revised the manuscript. Tiffany Guilleminot performed experiments and revised the manuscript. Dr Jacques Fourgeaud, Dr Marianne Burgard, Dr Slimane Allali, Prof. Julie Toubiana, Dr Yaël Pinhas, Prof. Pierre Frange and Dr Neil Derridj critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data availability

The Parvovirus B19 sequences are available in GenBank with accession numbers (PP951097, PP951098, PP951099, PP951100, PP951101, PP951102, PP951103, PP951104, PP951105, PP951106, PP951107, PP951108, PP951109, PP951110, PP951111, PP951112, PP951113, PP951114, PP951115, PP951116, PP951117, PP951118, PP951119, PP951120, PP968059, PP968060 and PP968061). Raw data are available upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106409.

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