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Emergence of measles virus genotype D8 amidst endemic B3 circulation in Uganda, 2023-2025

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ABSTRACT

Objectives: Measles remains a significant global health threat, with recurrent outbreaks, even in regions such as America, where the disease had been eliminated. Despite the availability of an effective and freely accessible vaccine, measles remains a global burden especially in children. The World Health Organization (WHO) estimated 107,500 measles-related deaths in 2023, with over 95% occurring in low- and middle-income countries reflecting fragile health systems. Uganda has similarly experienced persistent outbreaks since 2023. This study aimed at investigating the emergence of measles genotype D8 in Uganda for the first time, alongside the endemic measles genotype B3.

Methods: To track measles circulating strains, molecular genotyping targeting the measles virus N450 region was performed on 137 specimens. Phylogenetic analysis of the sequences was carried out along with WHO reference sequences and GenBank selected sequences.

Results: Alongside the endemic B3 genotype, genotype D8 was detected for the first time in Uganda, revealing co-circulation of both lineages.

Conclusions: The detection of measles D8 alongside the endemic B3 indicated possible gaps in the existing surveillance system and vaccination coverage. These findings highlight the critical importance of molecular surveillance and the urgent need to enhance measles vaccination coverage.

Introduction

Measles is a highly contagious viral disease, with a single infected person capable of transmitting the virus to at most 60 susceptible individuals during close contact [1]. Despite the availability of an effective live attenuated vaccine, measles has resurged globally including in regions that had previously attained elimination, such as the Americas [2]. Approximately 20% of unvaccinated individuals with measles require hospitalization, with 5% developing pneumonia and 0.01% progressing to encephalitis; a potentially disabling condition that can result in deafness, seizures, or cognitive impairment [3]. Hence, measles remains endemic in several World Health Organization (WHO) regions, including the African Region (AFRO), South-East Asia Region (SEARO), and Eastern Mediterranean Region (EMRO) where transmission continues year-round with periodic outbreaks. In 2024 alone, the European region reported 127,350 cases of measles, which was the highest since 1997 [4].

Uganda is no exception. Since 2023, the country has experienced uninterrupted measles outbreaks including the detection of non-endemic virus strains. Persistent measles transmission in Uganda is largely attributed to suboptimal vaccination coverage with measles-containing vaccines (MCVs) at both national and sub-

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national (district) levels. Although WHO/UNICEF Estimates of National Immunization Coverage (WUENIC) reported an increase in MCV1 coverage from 79% in 2016 to 93% in 2023, the Uganda Demographic and Health Survey (UDHS) 2022 revealed consistently lower coverage across all antigens. Notably, MCV2, introduced in 2022 achieved only 50% annualized administrative coverage by 2024 [5]. There was a general global trend in the reduction of vaccination coverages across all antigens following the COVID-19 global pandemic [6]. These gaps contribute to the accumulation of susceptible individuals and fuel recurrent outbreaks, posing a significant threat to Uganda's measles elimination goals. WHO recommends achieving ≥95% coverage with two MCV doses at national and sub-national levels to eliminate measles which would provide approximately 97% effectiveness in preventing both infection and serious complications. Monitoring progress toward this goal requires robust molecular surveillance to distinguish endemic from imported measles virus strains.

In Uganda, molecular surveillance has consistently identified genotype B3 as the endemic strain for nearly 2 decades [7,8]. Globally, 24 measles genotypes have been described but immunization campaigns have reduced circulating genotypes to fewer than five, with B3 and D8 remaining the most prevalent [9]. Correct interpretation of sequence data is essential in public health so that chains of transmission and immunization gaps are identified early, and control measures be implanted in a timely manner to prevent further disease spread. This study reports the first occurrence of measles genotype D8 in Uganda and details the concurrent circulation of genotype B3 which will inform the immunization program of the necessary action of mass campaigns.

Materials and methods

Patient demographics

The Measles National Laboratory in Uganda analyzed data from suspected measles cases recorded in the national laboratory and case-based surveillance databases between January 2023 and April 2025. Data reviewed included patient demographics, clinical history, and laboratory results.

Sample collection and measles virus immunoglobulin (Ig) M antibody detection

Blood specimens were collected by health workers from patients presenting with fever and rash, and one or more of the following symptoms: cough, coryza, or conjunctivitis. Samples were sent to the Measles National Laboratory at the Uganda Virus Research Institute for diagnosis. Measles IgM testing was performed using WHO-approved enzyme-linked immunosorbent assay kits (Euroimmun or Virion Serion) according to the manufacturers' instructions. A confirmed measles outbreak was defined as ≥3 IgM-positive cases from the same district within 30 days. Following outbreak confirmation, oral fluid and throat swab samples were collected from IgM-confirmed cases within 28 and 5 days from rash onset respectively. Throat swab samples were placed in virus transport media (VTM) and transported on frozen ice packs to the National Measles Laboratory within 24 hours for genotype identification and characterization.

Sample processing, measles virus detection, amplification, and sequencing

Samples were processed according to WHO guidelines [5] and stored at −80°C. Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) targeting the N gene was performed following WHO guidelines and primers as described by Hummel et al. [5,10]. Samples positive on the qRT-PCR were subjected to a genotyping PCR using the Qiagen OneStep PCR Kit (Thermo Fisher Scientific, USA) and primers developed by Bankamp et al. [11,12]. Samples producing the expected 634 bp amplicon (coding for the 3′ terminus of the N gene) were visualized on 2% agarose gel. RT-PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific, USA) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Genotypes were assigned based on analysis of the 450 bp partial coding region of the N gene.

Data analysis

Descriptive statistics were generated using Stata 14 (StataCorp LLC, College Station, TX, USA). Percentages were calculated based on IgM-positive or IgM-negative results, while borderline IgM results and instances where IgM testing was not done were excluded from cross tabulations. Genotype distribution maps were created using RStudio (Posit Software, Boston, MA, USA).

Phylogenetic analysis: (N-450)

Raw sequences were trimmed and base-called using the web-based tool Recall v2.35.15 [13]. All outbreak sequences were named according to WHO nomenclature [14]. The N-450 base pair sequences were aligned with the 24 WHO reference strains, and the identified genotypes were further compared with selected measles virus strains from National Center for Biotechnology Information (NCBI). Multiple sequence alignment of N-450 consensus sequences was performed using the MAFFT v7. Phylogenetic trees were generated in IQ-TREE v2.4.0 using the Tamura–Nei + Fixed (TN+F) substitution model and the maximum likelihood method. Tree reliability was assessed with 1,000 bootstrap replications. Tree annotation was done using the Interactive Tree of Life (iTOL) v7.2.2.

Field epidemiological investigation of D8

Having detected the first D8 in Uganda through phylogenetic analysis, the team from the laboratory, followed up to where the first D8 case had been reported. Interviews were held with the guardian of the first reported case of D8 to ascertain the possible source. In addition, the neighboring areas within the district were surveyed for any possible uninvestigated cases.

Results

Between January 2023 and April 2025, 4616 suspected measles cases were investigated across Uganda. Of these, 1412 (30.6%) were confirmed positive for measles IgM antibodies. The number of confirmed cases showed a gradual increase over the study period, peaking in January 2025 as indicated in Figure 1.

Demographics, vaccination status, and coverage

The gender distribution was relatively balanced, with 735 males (52.1%) and 677 females (48.0%). Children aged 1-4 years (679) were the most affected group, representing 48.1% of confirmed cases. They were followed by infants aged <1 year at 26.1% (368), of whom 302 (21.4%) were aged ≤9 months.

Of the confirmed measles cases, 175 (12.4%) had evidence of vaccination. In contrast, 1,237 (87.6%) of the confirmed measles cases had missing vaccination information with 571 (17.1%) being ≤9 months (not eligible for vaccination). Among vaccinated cases, 151 (86.3%) had received a single dose of measles rubella vaccine, while 24 (13.7%) had received ≥2 doses as indicated in Table 1.

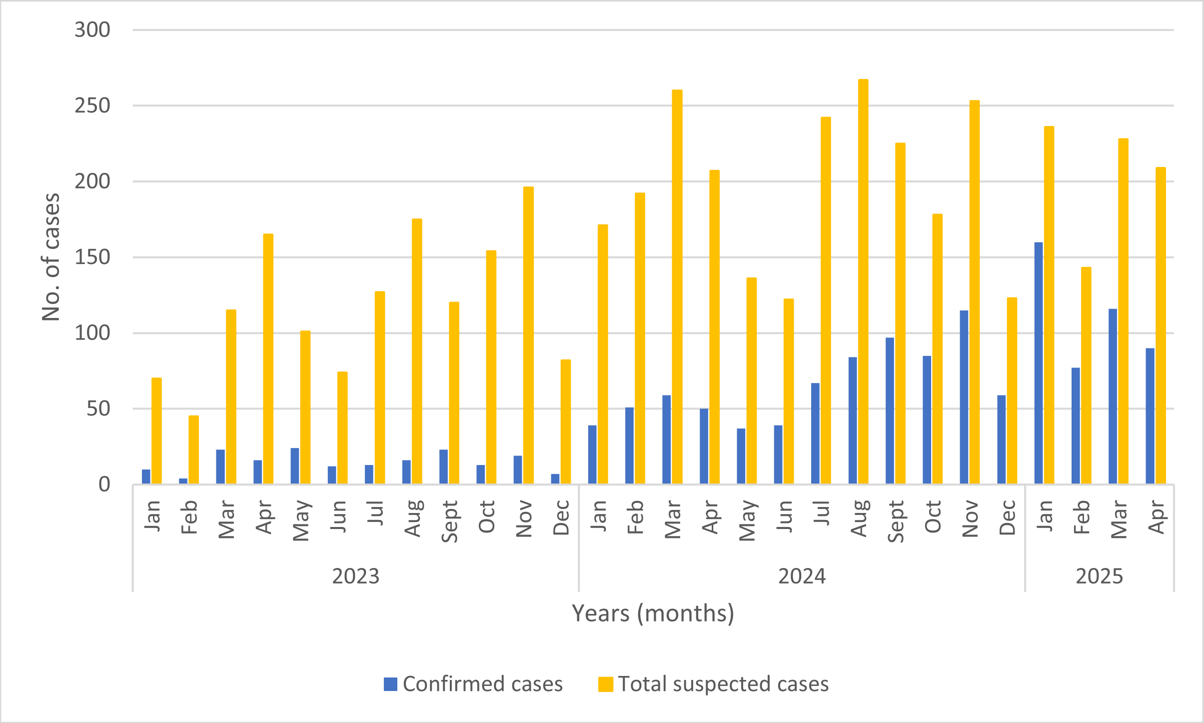

Figure 1. Suspected measles cases and their corresponding immunoglobulin M results. This graph shows the total number of investigated suspected measles cases and their corresponding laboratory immunoglobulin M results from January 2023 to April 2025.

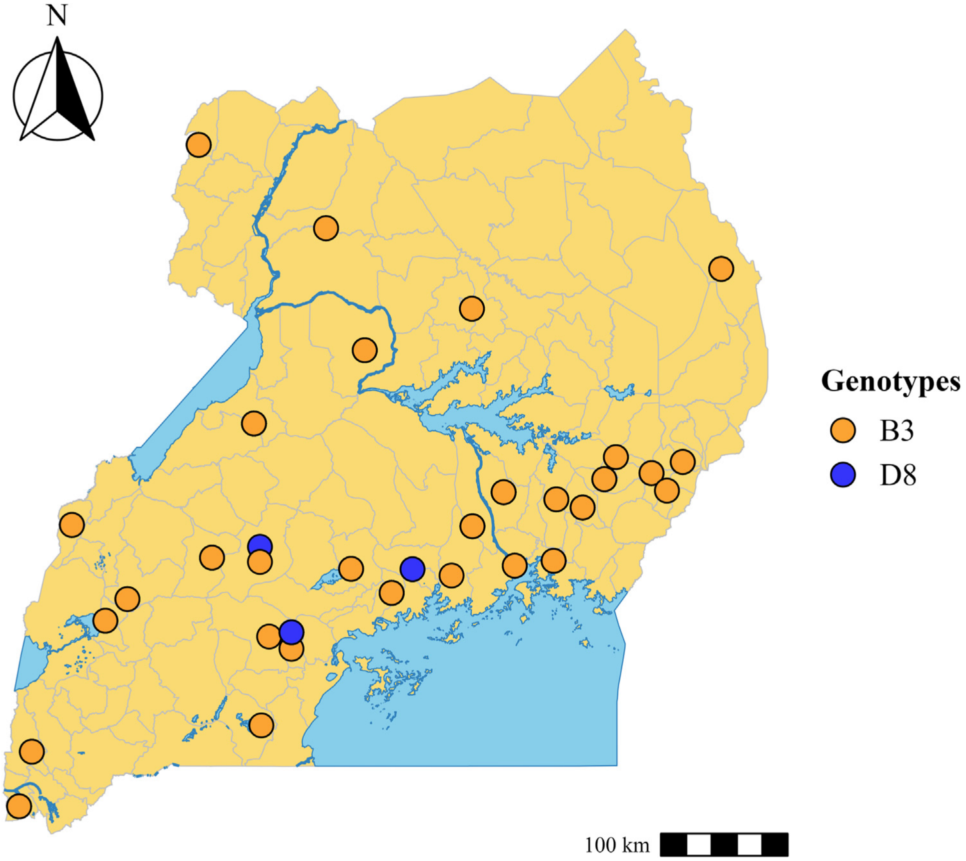

Figure 2. Geographic distribution of measles virus genotypes in Uganda. This map illustrates the spatial distribution of Measles virus genotypes from January 2023 to April 2025. Each circle within the map represents a measles virus genotype in a particular district (B3; orange, D8; blue).

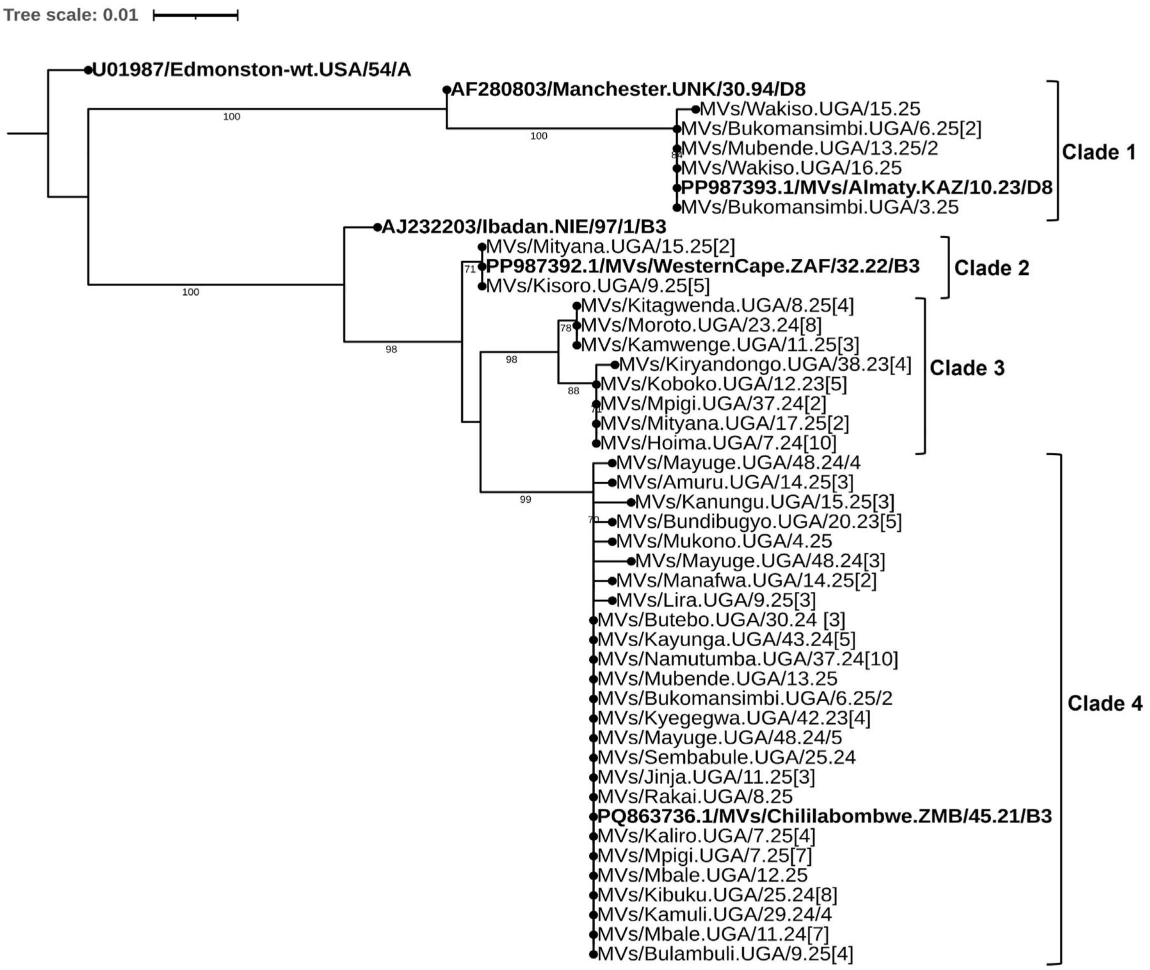

Figure 3. A phylogenetic tree showing measles virus strains in Uganda, representing two co-circulating genotypes; B3 & D8. The phylogenetic tree was calculated using the maximum likelihood method and TN+F parameter model, using 1000 bootstrap replications. The numbers in brackets '[]' indicate the number of 100% identical sequences from the same outbreak and district. GenBank (MVs/Almaty.KAZ/10.23, MVs/WesternCape.ZAF/32.22, MVs/Chillabombwe.ZMB/45.21) and WHO reference sequences (AF280803/Manchester.UNK/30.94/D8, AJ232203/Ibadan.NIE/97/1/B3 & U01987/Edmonston-wt.USA/54/A) included in the tree are shown alongside their WHO names in bold.

D8 epidemiological investigation results

A follow-up investigation for the first case of the D8 measles genotype in Bukomansimbi district unearthed that the home had been visited by a relative (alleged index case) from Wakiso district who manifested with a rash. However, other samples collected far away from the first case during the same follow up in Bukomansimbi identified another two patients with the D8 measles genotype. Further follow-up done in homes in Wakiso identified two patients with the D8 measles genotype, while routine surveillance identified one patient with the D8 measles genotype in the Mubende district.

Genotype distribution and detection of D8

Of the 137 measles virus samples that underwent molecular testing, 131 (95.6%) belonged to genotype B3, while six (4.4%) belonged to genotype D8. The first D8 case was detected in Bukomansimbi district with subsequent surveillance identifying additional cases in Bukomansimbi, Wakiso (2 epidemiologically linked cases), and Mubende (1 case) districts suggesting widespread circulation of this genotype within the central region of Uganda as shown in Figure 2.

Phylogenetic analysis and clade characterization

The Uganda measles virus sequences between 2023 to April 2025 were grouped into four major clades (Figure 3). Clade 1 had D8 sequences, four of which were 100% identical to the sequences from Kazakhstan. Clade 2 had sequences identical to the strain from South Africa. Clade 3 formed a cluster consisting of only Ugandan sequences. The earliest of these sequences were from Koboko district. However, these sequences were obtained from individuals from the Republic of South Sudan (RSS) residing in Koboko at the time of sample collection. Clade 4 had mostly Ugandan sequences, which were identical to the strain from Zambia, with a few other sequences forming genetically distinct subgroups within the cluster. In addition to the existing strains from 2023 to 2024, six new subgroups have been identified in 2025.

Measles genotype D8 sequences were further analyzed along with the global D8 sequences to assess for the possible source of importation as indicated in Figure 4. Four of the D8 sequences were identical to strains from Kazakhstan(2023), Italy (2024), and Bosnia and Herzegovina (2024). One D8 sequence did not cluster with the others, showing no close identity with other known sequences worldwide.

Measles case information from Uganda and Sequence data from this report were deposited into the MeaNS database

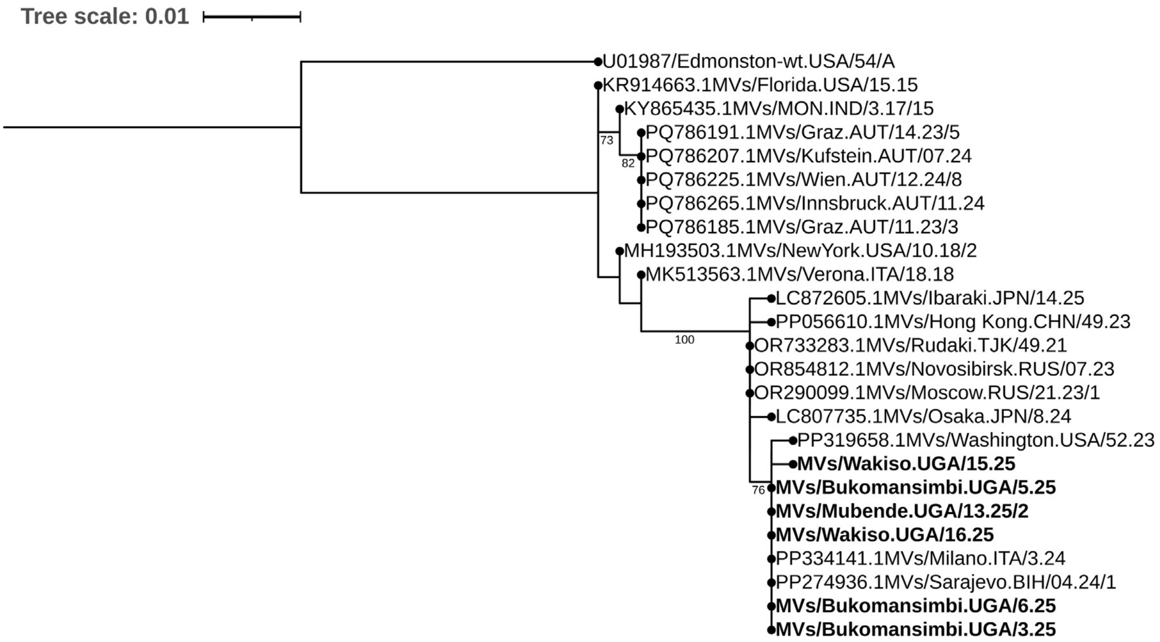

Figure 4. Phylogenetic analysis of the measles genotype D8 from Uganda with selected GenBank sequences. The phylogenetic tree was calculated using the maximum likelihood method and TN+F parameter model, using 1000 bootstrap replications. Gene bank accession numbers of sequences included in the tree are shown alongside their World Health Organization reference names, and the Ugandan test samples under investigation are in bold.

Table 1

Demographics, IgM, vaccination status (Table 1a) and number of days between rash onset and blood collection (Table 1b) among patients with suspected measles disease.

Table 1a

Characteristics	IgM Positive n, (%)	IgM Negative n, (%)	Total n, (%)
Sex			
Male	735 (52.1)	1,637 (52.7)	2,420 (52.4)
Female	677 (48.0)	1,469 (47.3)	2,196 (47.6)
Age[a]			
<1	368 (26.1)	388 (12.5)	771 (16.7)
1 - 4	679 (48.1)	1,612 (51.9)	2,329 (50.5)
5 - 9	212 (15.0)	773 (24.9)	1,005 (21.8)
10 - 14	58 (4.1)	217 (7.0)	288 (6.2)
15 - 24	59 (4.2)	74 (2.4)	139 (3.0)
25 - 40	27 (1.9)	28 (0.9)	60 (1.3)
>40	9 (0.6)	10 (0.3)	20 (0.4)
Vaccination status[b]			
Evidence of vaccination	175 (12.4)	1,086 (35.0)	1,280 (27.7)
Unvaccinated	-	-	-
Missing information	1,237 (87.6)	2,020 (65.0)	3,336 (72.3)
Number of vaccine doses[c]			
1	151 (86.3)	825 (76.0)	992 (77.5)
2	18 (10.3)	205 (18.9)	226 (17.7)
3	5 (3.0)	35 (3.2)	40 (3.1)
4	1 (0.6)	17 (1.6)	18 (1.4)
5	0 (0.0)	4 (0.4)	4 (0.3)

Table 1b

Days	IgM Positive (n)	IgM Negative (n)	Indeterminate (n)
0-3	836	1968	60
4-28	557	1075	28
>28	13	50	2

[a] The age of four patients was not recorded and therefore they were excluded from age categorization.

[b] Vaccination status was determined by the presence of the date of last vaccination.

[c] The number of vaccine doses amongst those with evidence of vaccination.

and the GenBank under accession numbers PX359058-PX359216 (Supplementary Table 1).

We identified, for the first time, the measles virus genotype D8 amidst the endemic circulation of genotype B3 in Uganda between 2023 and 2025, a period during which the Ministry of Health surveillance confirmed multiple outbreaks. To achieve elimination status, active surveillance, optimal routine immunization program, and continuous genetic characterization of circulating strains remains essential. Molecular surveillance not only tracks transmission pathways of measles genotypes but also enables the Global Measles Laboratory Network to monitor patterns, investigate origins, and inform elimination strategies. In this retrospective outbreak investigation, genotype B3 was circulating throughout the period between 2023 and 2025, whereas D8 was only detected in 2025 which indicated overlapping transmission chains between the endemic B3 and the newly detected D8. For genotype D8, four sequences matched strains from Kazakhstan (2023), and Bosnia and Herzegovina (2024). One sequence from Wakiso was genetically distinct, possibly due to undetected transmission. Although none of the D8 cases reported travel history, these findings suggest silent in-country circulation, likely introduced through cross-border spread. The detection of D8 alongside endemic B3 highlights persistent transmission gaps, uninterrupted disease spread that is also fueled by low vaccination coverage and vaccine hesitancy in communities [15,16]. A high proportion of cases in infants <1 year suggests waning maternal antibodies, raising the policy question of whether measles vaccination should be administered at 6 months during outbreak response. Furthermore, the detection of multiple B3 subgroups demonstrates ongoing viral evolution and reinforces the need for sustained molecular surveillance.

Globally, two measles virus genotypes B3 and D8 have been in circulation since 2021 [17]. However, molecular surveillance in Uganda detected a new genotype D10 in the early 2000s which has not been seen again since 2002 [18]. Subsequently, genotype B3 emerged and has remained the endemic strain since 2011 [7]. However, in January 2025, genotype D8 was confirmed in Uganda for the first time. Phylogenetic analysis showed that one distinct D8 virus strain diverged from previously known strains [19], suggesting evolution of undetected regional circulation. Other D8 sequences clustered with strains from Europe, including Italy (2024) and Bosnia and Herzegovina (2024) indicating possible importation. Whereas genotype B3 remains firmly established as endemic in Uganda, other etiologies that present with fever and rash need to be further investigated, since only 30% of the investigated samples were confirmed as measles IgM-positive. These findings are similar to those shared in 2024 by Namuwulya et al. [7,8]. Phylogenetic analysis of the B3 genotype revealed three clades. Clade 2, first detected in Uganda 2025, was identical to a South African strain, underscoring global exchange of measles viruses within the African region, further highlighting the uninterrupted regional and global measles circulation. Clade 3 was unique to Uganda, linked to border districts and refugee settlements, pointing to cross-border spread as samples picked from Koboko were all refugees from the RSS. Clade 4 had a majority of identical and a few closely-related sequences to a Zambian strain. This was the most predominant group of viruses, showing greater transmissibility with this strain picked up in all regions of Uganda. New subgroups continue to emerge within the B3 clades, with six additional lineages identified in 2025, consistent with ongoing mutations and active local transmission. For a while, the B3 genotype has also been known to be endemic in the African region, attributed to its high transmissibility [14,20]. However, the new emerging subgroups of B3 are indicative of genetic rearrangements that may be creating more virulent strains. In addition to the uninterrupted clade diversity, this data highlights the persistence and high measles burden in Uganda, despite long-standing vaccination efforts that have not achieved the required levels of herd immunity. Of particular concern, was a prolonged outbreak spanning from 2023 to 2025 with the most affected group being children aged 1-4 years; however, this group is the primary target of preventive interventions, such as routine measles vaccination. Overall, children aged 1–4 years accounted for 48.1% of confirmed cases, while infants under 1 year contributed 26.1%, a notably high proportion given that vaccination only begins at 9 months. Among these infants, 21.4% were infected before they were eligible for the first vaccine dose. These findings highlight that children, particularly those too young to be vaccinated, remain highly vulnerable to measles infection, requiring exposure to the vaccine in early childhood [21,22]. Among confirmed cases, a substantial proportion (87.6%) had no vaccination record, and only 12.4% had evidence of vaccination. Even among vaccinated individuals, 86.3% had received only a single dose, with very few completing ≥2 doses. This reflects Uganda's low uptake of the second MCV2, which stood at 54% by March 2025. This is below the ≥95% coverage needed for herd immunity, this being attributed to the persistent negative impact of COVID-19 toward vaccination coverage and response [23–25]. The absence of vaccination records further complicates the understanding of whether the consistent outbreaks are as a result of absence of vaccination or under vaccination.

Conclusion

Measles outbreaks have continued to occur in Uganda with a D8 genotype identified for a first time, amidst circulation of the endemic B3 genotype, both likely undergoing mutations. The two strains of the D8 genotype detected in Uganda between January and April 2025 indicate possible gaps in the existing surveillance system. The likely importation of D8 and the endemicity of B3 indicates low vaccination coverage in Uganda since over 70% of the cases are occurring in individuals with zero dose of measles-containing vaccine.

Recommendations

To interrupt measles transmission, Uganda must achieve and sustain ≥95% coverage with both MCV1 and MCV2 nationwide. Cross-border and refugee camp surveillance and vaccination measures should be strengthened to monitor viral introductions. Vaccination documentation systems must be improved to provide more information for accurate epidemiological classification. Finally, policymakers should consider reviewing the timing of the first measles vaccine dose in high-transmission settings to better protect infants who are most at risk. In addition, as countries advance toward measles elimination, emphasis on collecting molecular specimens among clinically suspected cases at the same time as blood is essential for timely molecular surveillance.

Declaration of competing interests

The authors have no competing interests to declare.

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Ethics approval

This work represents ongoing routine surveillance work and therefore no additional approval was obtained.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108255.

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