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Genetic characterization of measles virus circulating in Iran, 2021–2023

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Abstract

Background Measles, an ongoing public health concern, demands continuous molecular surveillance and virus characterization for elimination. Despite Iran achieving measles elimination status in 2019 through robust molecular testing and vaccination, the COVID-19 pandemic disrupted global vaccination efforts, leading to increased measles-related morbidity and mortality. This study aims to overview measles virus serological and molecular traits in Iran from 1st January 2021 to 30th April 2023.

Methods Following World Health Organization and Center for Diseases and Control protocols, serological tests were performed on suspected cases and the nucleoprotein (N) gene of confirmed cases were subsequently amplified using molecular methods and were sequenced afterwards for measles genotyping. Phylogenetic analysis was performed with the obtained sequences.

Results Analyzing 17,343 suspected cases from 1st January 2021 to 30th April 2023, 936, 177, and 164 samples were positive using ELISA, quantitative Reverse transcription PCR, and Reverse transcription PCR, respectively. The B3 genotype predominated, notably in Iran's South East (41%), Central (28%), and South (13%) regions. Provinces bordering countries with measles outbreaks exhibited higher risk of virus importation. Genetic comparisons with measles sequences submitted to NCBI and MeaNS databases revealed direct importation and contact transmission.

Conclusion Regular surveillance and genetic analysis are critical for understanding measles transmission and reacting to outbreaks. The COVID-19 pandemic yielded mixed effects on measles cases, enhancing hygiene measures while causing underreporting and vaccination gaps. Vigilance against measles resurgence is crucial, requiring cross-border transmission studies, improving cross-border surveillance and adaptable vaccination strategies.

Keywords Measles, Molecular surveillance, Genotypic characterization, COVID-19, B3 genotype, Iran

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Introduction

Measles, a highly contagious Paramyxovirus, causing fever, rash, and other symptoms, remains a global public health concern, particularly among children [1, 2]. While cases decreased from 853,479 in 2000 to 132,490 in 2016, they surged to 869,770 with 207,500 deaths in 2019 [3]. In 2022, cases rose by 18% to 9 million, with 136,000 fatalities, primarily due to declining vaccine coverage [4]. The virus exhibits eight clades (A to H) and 24 genotypes based on the nucleoprotein (N) gene (N-450) [5]. In 2003, 18 co-circulating genotypes were identified. Since then, the number of genotypes has rapidly declined, and since 2021, only two genotypes—B3 and D8—has been detected [6]. Achieving and sustaining measles elimination status necessitates a comprehensive understanding of the virus's transmission dynamics and genotypic characteristics.

Molecular surveillance and genotypic characterization of the measles virus are pivotal in monitoring and responding to outbreaks. These approaches enable the identification of potential sources and routes of transmission. Before 2003, genotype D4 was the predominant measles strain in Iran. After the MR campaign in 2003, surveillance between 2002 and 2010 showed D4 remained the most common genotype, with H1 introduced from Southeast Asia [7, 8]. By 2010–2012, new strains like D8 and B3 were detected, particularly in regions near Pakistan and Afghanistan, suggesting the importation of measles from neighboring countries [9]. These findings underline the persistent challenges of measles control and surveillance despite vaccination efforts.

By employing molecular tests since 2002 and bolstering these efforts through extensive measles vaccination campaigns, Iran successfully achieved elimination status in 2019 and 2022 [10–12].

However, the onset of the COVID-19 pandemic disrupted vaccination programs globally, leading to a concerning resurgence of measles cases, accompanied by increased morbidity and mortality [13]. This situation highlights the importance of examining measles epidemiology during the pandemic to understand COVID-19's impact.

Given the proximity of certain provinces to neighboring countries reporting measles outbreaks, such as Afghanistan [14, 15], Pakistan [14, 16–18], and Armenia [14], there is an elevated risk of measles virus importation into Iran. Consequently, genetic comparisons with sequences from various sources, including genetic databases like NCBI and MeaNS, offer critical insights into imported cases and transmission routes.

This study aims to characterize the measles virus circulating in Iran during 2021–2023, with a focus on molecular surveillance and the impact of COVID-19 on the number of measles cases. This investigation included 17,343 suspected cases, analyzing positive samples using serology tests (ELISA), quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR), and Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Interestingly, the B3 genotype became the only strain in circulation and showed a high frequency in particular parts of Iran.

Materials and methods

A summarized diagram of the research workflow is presented in Fig. 1.

Clinical sample collection

Samples utilized in this study were kindly provided by the National Measles Laboratory (NML) of Iran. These samples belonged to patients, suspected of measles, presenting with fever and rash in Iran between January 2021 and April 2023. The collected specimens included throat swabs, urine, and serum samples.

Serology

Serum from suspected measles cases were analysed using the IgM ELISA test. The EUROIMMUN Anti-Measles Virus NP ELISA IgM kit (EUROIMMUN AG, Germany) was employed to assess the serum during this period. Positive IgM samples (throat swabs, urine) were collected and subsequently subjected to molecular testing. Moreover, if a rise in IgM antibody levels of a vaccinated case is described as "positive" (within 2 months after vaccination), then it would be regarded as a "recently vaccinated" case, and no further molecular testing is performed.

Molecular analysis

Molecular analysis was conducted on serum-positive and serum-negative samples from individuals whose serum was collected within 72 h of rash onset. RNA was extracted from clinical specimens using the High Pure Viral RNA Kit manufactured by Roche GmbH in Germany. Subsequently, qRT-PCR was conducted using the 4X CAPITAL™ 1-Step qRT-PCR Probe Master Mix (BiotechRabbit GmbH, Germany). Positive RNA samples were then selected for RT-PCR. The cDNA was synthesized and amplified using the Qiagen One-Step RT-PCR kit (QIAGEN, Germany) and the BiotechRabbit 1-step RT-PCR kit (BioRabbit GmbH, Germany), along with a set of primers (MeV 214 & MeV216) specific for the measles virus nucleoprotein (N) gene (C-terminal), according to WHO and CDC guidelines.

Approved PCR products (634 kb) were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced using an ABI 3130 Genetic Analyzer (Applied Biosystems, USA). The resulting sequences were

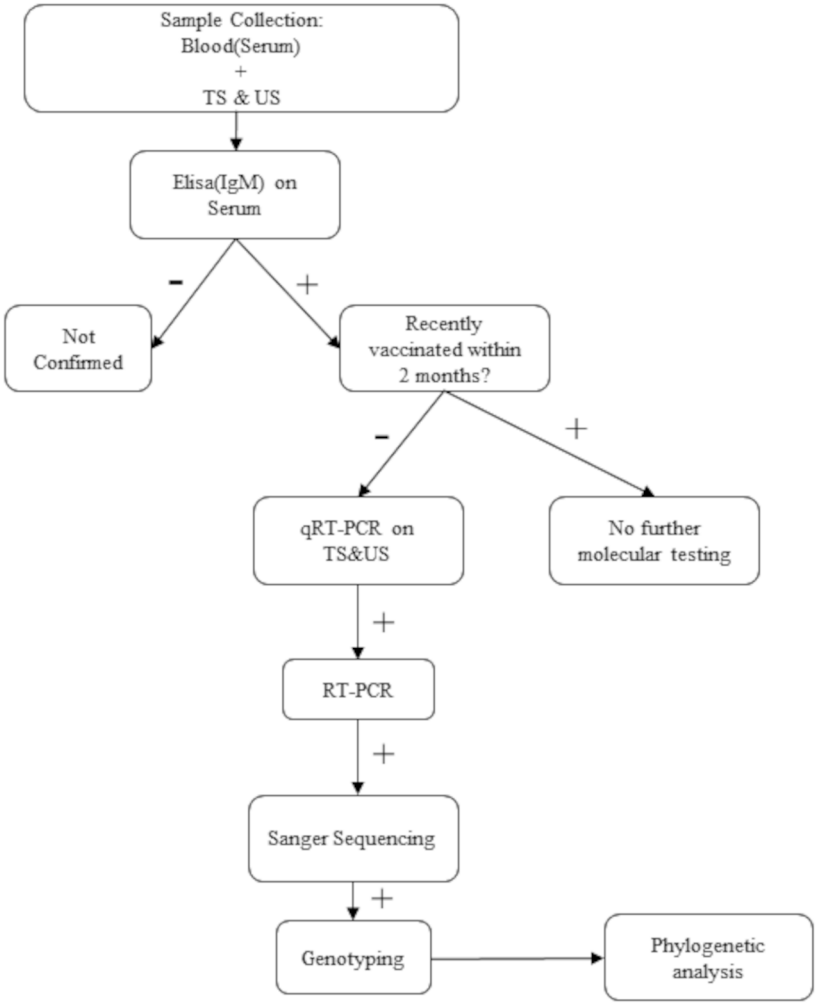

Fig. 1 A summarized workflow of sample collection, serological and molecular testing (TS = Throat Swab/ US = urine sample)

analyzed using the BLAST tool to determine the measles virus genotype.

Phylogenetic analysis

The acquired sequences were aligned using the Clustal W application within the BioEdit software. MEGA X was used for phylogenetic analysis with a maximum likelihood algorithm using K2 + G substitution model and a bootstrap value of 1000. Genotypes of the measles virus circulating during this period were represented in an unrooted phylogenetic tree using FigTree software (Fig. 4). The phylogenetic analysis included available WHO named-strain sequences and sequences from various countries submitted to the MeaNS and NCBI nucleotide database according to similarity percentages with the sequences in this study, aiding conclusions about virus importation.

Branch reliability and robustness were assessed through bootstrap analysis with 1000 iterations. Sequence nomenclature was submitted to MeaNS and GenBank databases, according to WHO recommendations. Accession numbers are provided in Table S1.

Results

Measles surveillance data

Suspected and confirmed measles cases

Based on serological testing using the IgM-ELISA technique, 5.39% ($n = 936/17,343$) of the total suspected cases (17,343) with rash and fever were confirmed as measles cases (Fig. 2). About 40% ($n = 371/936$) had been recently vaccinated, meaning they were not eligible for further

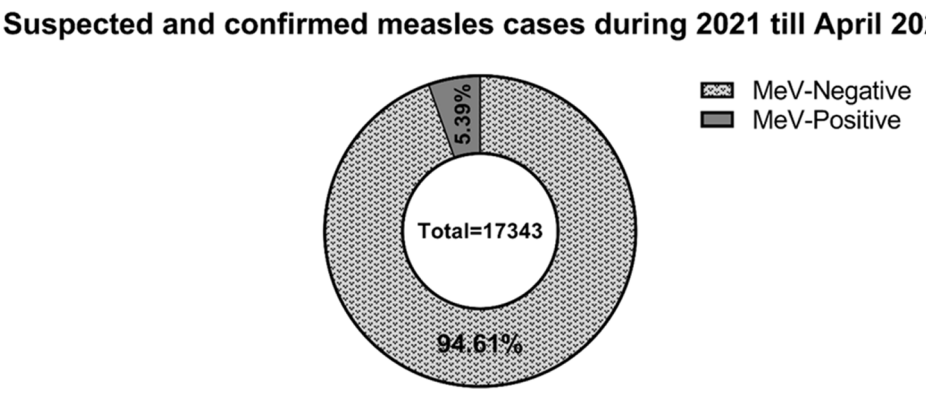

Fig. 2 Number of suspected & confirmed measles cases during 2021–2023

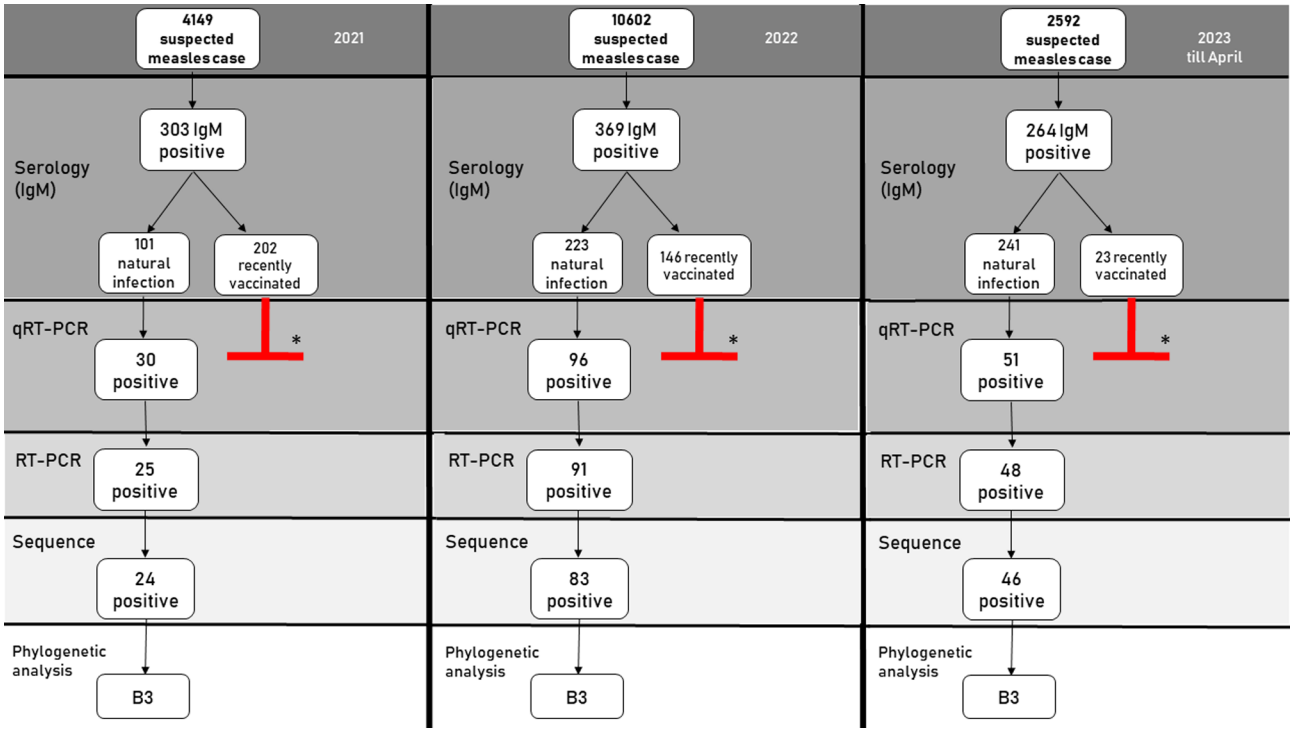

Fig. 3 Diagrammatic representation of the sequential procedure of the study results from the total number of suspected measles cases to phylogenetic analysis of the obtained sequences. *=the red shape implies that for recently vaccinated cases (cases with IgM positive result which have received vaccine (1^{st} or 2^{nd}) in two months prior to rash) no further molecular observation would be conducted are discarded from molecular observation

molecular testing (Fig. 3). The annual distribution of suspected and confirmed measles cases is also shown in Fig. 3, together with RT-PCR, qRT-PCR, and sequencing data.

In terms of serum-positive samples (excluding recent immunizations), 29.7% ($n=30/101$) in 2021, 43% ($n=96/223$) in 2022, and 21.16% ($n=51/241$) in 2023 tested positive using qRT-PCR. Furthermore, 83.33% ($n=25/30$) of the positive qRT-PCR samples tested positive using RT-PCR in 2021, 94.79% ($n=91/96$) in 2022, and 94.11% ($n=48/51$) in 2023. Additionally, sequencing results showed that 96% ($n=24/25$) of the positive RT-PCR samples in 2021, 91.2% ($n=83/91$) in 2022, and 95.83% ($n=46/48$) in 2023 were appropriately genotyped (Fig. 3).

Four of the 153 sequences were isolated from samples cultured in the Vero-hSLAM cell line, while the remaining 149 were taken directly from clinical samples.

In the following sections, the demographic characteristics of the cases will be discussed in greater detail.

Age and gender

In order to analyze the demographic characteristics of the cases, the age range for confirmed measles infections was one month to 79 years, with a median age of 5.25. According to the age distribution, nearly 50%(443/936)

of cases were in the 1–2-year age range; the high number of measles cases being in 1–2 year age group, especially in outbreak prone areas such as southeast and south regions, indicates that amid difficulties associated with those regions, immunization efforts were made in order to maintain the high vaccination coverage in Iran. This study highlights the significance of immunization in a susceptible age group. Patients' gender proportions, with a male-to-female ratio of 1.1:1, did not differ substantially.

Vaccination and region

According to vaccination history data, 52%(496/936) of confirmed patients were vaccinated, 33%(303/936) were not vaccinated, and 15%(137/936) had an unknown vaccination status. The number of measles cases with vaccination history implies a sufficient vaccination coverage in country. Between 496 confirmed cases of measles which had been vaccinated, 371 cases were identified as "recently vaccinated", meaning that they have received MMR vaccine within 2 months before clinical symptoms. When analysing the cases that were not vaccinated, it is observed that nearly 40% (117/303) were below 1 years old and thus could'nt receive MMR vaccine according to national immunization program. This data also supports that the vaccination coverage in Iran is sufficient.

Five isolated cases were also found to be immigrants from Afghanistan. These cases indicate the importation of measles virus from neighboring countries, with low vaccination coverage against measles, into Iran and could be as a result of illegal transportation of Afghan immigrants from Afghanistan to South East region of Iran. Regionally categorized sociodemographic data is shown in Table S1.

Phylogenetic analysis and genotype characterization

All 153 measles sequences in this study belonged to the B3 genotype. The phylogenetic tree encompassing all sequences across the years is depicted in Fig. 4. Most of the sequences belonged to cluster B3.3, while several sequences were grouped in B3.1 and B3.2 clusters. While clusters B3.2 and B3.1 were only discovered in 2022, cluster B3.3 was consistently detected in every year from 2021 to 2023. The measles reference strains MVs/Islamabad.PAK/1.13 and MVs/Quetta.PAK/44.20, both of which are from Pakistan, were significantly similar to sequences from cluster B3.3, indicating the possibility of cross-border transmission. Additionally, cluster B3.1 sequences showed similarities to the WHO named-strain MVs/Oslo.NOR/16.18. The nucleotide similarity between cluster B3.3 sequences and the B3 genotype reference sequence (IBADAN.NIE/97/B3) varied from 95.7 to 97.5%, reflecting the nucleotide variation between them. In neighboring countries such as Pakistan and Afghanistan, the dominant genotype during this period was B3 genotype. As a result of illegal immigration and transportation from this two country and because of cultural differences in southeast and south regions of Iran (high number of children, living in large families), the B3 genotype was the only genotype detected.

Individual phylogenetic trees for each year (2021, 2022, and 2023) are illustrated in the S1 Figure, S2 Figure, and S3 Figure. Average sequence similarity with the measles reference sequences IBADAN.NIE/97/B3 and NY.USA/94/B3 is 97.16% and 96.1%, respectively.

Discussion

Measles, an immensely contagious viral infection caused by the measles virus, remains a significant global public health threat, particularly affecting the vulnerable demographic of young children and unvaccinated adults. Despite substantial advances in vaccination campaigns, the declaration of measles elimination in Iran in 2019 [10] did not provide an invulnerable barrier against the re-emergence of outbreaks in different regions of the country.

According to unpublished data (Table S2), the number of confirmed measles cases, evaluated by techniques such as IgM-ELISA and RT-PCR, reached a low in 2020, with just three confirmed cases (IgM-ELISA) and no positive viral isolation or molecular testing.

However, 2021 to 2023 showed a different picture, with an increase in the number of cases as a result of interrupted vaccination during the pandemic period. This resurgence could be attributed to the widespread implementation of stringent hygiene protocols, such as mask use and social distancing, and the temporary closure of public facilities like schools due to COVID-19 [19–21]. While these preventive measures were crucial in reducing virus transmission, they may have unintentionally triggered changes in measles epidemiology.

Amidst the global COVID-19 epidemic, global vaccination campaigns faced obstacles from the logistical difficulties caused by widespread lockdowns. This administrative delay may have led to an underestimation of measles cases, worsened by a decline in vaccination rates caused by public concerns over COVID-19 [13, 22–25]. Furthermore, the majority of measles cases are reported in Iran's southeast, central, and south regions. Illegal immigration from Pakistan and Afghanistan, both of which saw several measles outbreaks during the COVID-19 epidemic [16, 17, 20, 26, 27], is one contributing reason. This emphasizes how crucial cross-border surveillance is in regions where outbreaks are likely to occur because of these factors. The migration wave from Afghanistan in 2021 and the region's inadequate measles vaccination coverage appeared to be an additional factor

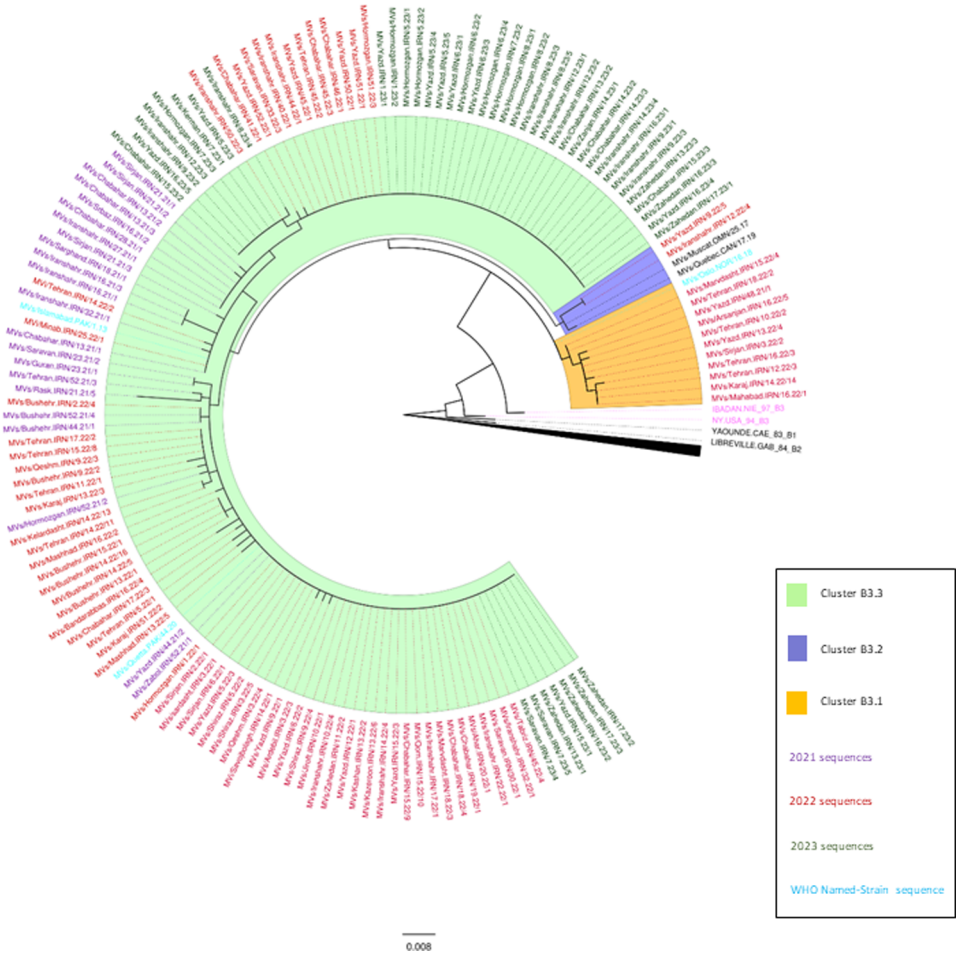

Fig. 4 Phylogenetic analysis of Iranian measles sequences (B3) using the N-450 region during 2021–2023 alongside B3 reference sequences and WHO named-strains using FigTree software. 2021 strains are displayed with purple colour, 2022 strains are displayed with red colour and 2023 strains are displayed with green colour. WHO named-strains (MVs/Islamabad.PAK/1.13, MVs/Quetta.PAK/44.20 and MVs/Oslo.NOR/16.18) are displayed with blue colour. MVs/Muscat.OMN/25.17 and MVs/Quebec.CAN/17.19 have the most similarity with cluster B3.2 sequences and are shown in black colour. B3 reference sequences are displayed with pink colour. Reference sequences belonging to other measles genotypes are compressed and shown by dark triangle

for measles transmission and a likely route for the virus's entry into Iran [28, 29].

Despite obtaining an impressive MCV2 coverage of equal to or more than 95% in 2017 [30], the increasing rate of confirmed cases between 2021 and 2023 has raised concerns over the sustainability of vaccination coverage. This might result in falling below the threshold needed to develop herd immunity against measles [31]. Of particular significance is the prevalence of cases in the 1–2-year age group (47.32%; $n = 443/936$) and the high percentage of cases (49.74%; $n = 123$) involving newborns under one year of age in 2023. The fact that the majority of measles cases occur in children aged 1–2 years reflects a high vaccination rate, as the first vaccine dose is given at 12 months and the second at 18 months. Because the MMR vaccine is a live attenuated vaccine, the symptoms may be less than those caused by the illness itself. However, the fact that the majority of cases in 2023 were in infants under one year old emphasizes the need of monitoring vaccination regimens in places facing measles outbreaks.These findings show the need to reconsider vaccination timing. Previously, the immunization age for Iranian children was 9–15 months. The time of vaccination was decided to be 12 months due to changes in the epidemiology of measles incidence. Still, vaccine administration for children living in susceptible regions before 12 months of age would cause efficient outbreak control and higher vaccine coverage, resulting in herd immunity [27, 32–34]. The investigation of providing vaccinations to children in outbreak-prone areas before the regular 12-month threshold gains importance and needs further justification, enhancing the efficacy of outbreak control measures and achieving herd immunity.

Moreover, implementing multi-dose vaccination strategies during outbreaks could potentially serve as a disruptive mechanism to restrict viral transmission. Although, the MMR vaccine contains the live attenuated strain of Edmonston strain (genotype A), reports are indicating

diminished efficacy of neutralizing antibodies produced against the B3 genotype which significantly questions the efficiency of measles vaccination [31, 35, 36] which needs further exploration. The potential role of maternal antibodies derived from the Edmonston vaccine strain, belonging to genotype A, in the elevated incidence rate among infants below one year old emphasizes the viability of considering a vaccine strain switch to counteract the circulation of the B3 genotype.

While B3,H1 and D8 genotypes were the most common worldwide measles genotype in circulation until 2018, B3 and D8 genotypes are still surging in 2024, however, the latest H1 genotype report was in september 2019, indicating a possible inactivation of the genotype in its prevalence [37, 38]. The persistence of the B3 genotype, first detected in Iran in 2012 [9], clearly suggests its introduction from outside sources, casting doubt on its endemic status. Phylogenetic analyses that relate B3 genotypes to strains from neighboring countries provide valuable insights into probable importation sources. The sequences during these outbreaks had considerable similarities (up to 100%) to WHO named-strains MVs/Islamabad.PAK/1.13,MVs/Quetta.PAK/44.20,MVs/Oslo.NOR/16.18 and MVs/Bradford.GBR/13.18. Therefore, it could indicate possible cross-border transmission events, necessity of further virus evolution monitoring, need for improved public health responses such as vaccination campaigns and underlying epidemiological links between outbreaks. However, a comprehensive investigation necessitates applying advanced techniques such as the Molecular Clock, MF-NCR, and Whole-Genome Sequencing (WGS) methods to accurately trace the virus's origin and delineate transmission routes [39]. Notably, the higher Reproduction number (R0) attributed to the B3 genotype accentuates its rapid transmission dynamics [40].

This study highlights the critical need for ongoing genotypic analysis and molecular surveillance in understanding the complex dynamics of measles transmission, especially in light of the novel challenges brought on by the COVID-19 pandemic. Ongoing outbreaks of the B3 genotype highlight how critical it is to maintain constant surveillance, conduct careful monitoring, and implement focused preventative approaches to prevent measles outbreaks.

Conclusion

In conclusion, this study provides insights into the molecular basis and epidemiological trends of the measles virus in Iran from January 2021 to April 2023. The findings shed understanding on the continued viral transmission within Iran's borders, highlighting the critical significance of effective vaccination campaigns in preventing its spread and closing possible vaccination gaps and also enhancing cross-border surveillance efforts. Promoting the use of advanced molecular techniques like whole-genome sequencing (WGS) and molecular clock would give us the benefit to perform in-depth analysis of measles transmission routes and origin. Effective measles outbreak management and prevention, both inside Iran and globally, continue to rely on a persistent commitment to vigilant viral surveillance and constant monitoring. An unrelenting commitment to public health takes center stage to manage these difficulties, moving us toward a future where measles transmission is no longer a problem (See Table 1).

Table 1 An overview of study key findings

Key findings
• Ascending numbers of measles cases from 2021 to 2023 in comparison to previous years
• B3 genotype as the only genotype detected
• Most cases were detected in SouthEast (41%), Central (28%) and South(13%) regions
• Cross-border transmission possibility of measles virus in regions neighboring countries with recurrent measles outbreak
• Possible effect of COVID-19 pandemic on vaccination coverage and resulting outbreaks

Abbreviations

COVID-19	Coronavirus disease 2019
ELISA	Enzyme-Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
TS	Throat Swab
US	Urine Sample
NML	National Measles Laboratory
hSLAM	human Signaling Lymphocytic Activation Molecule
MMR vaccine	Measles, Mumps and Rubella vaccine
MCV2	Second-dose of Measles Containing Vaccine
MF-NCR	Non Coding Region between Matrix and Fusion
WGS	Whole Genome Sequencing
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
NCBI	National Center for Biotechnology Information
MeaNS	Measles nucleotide surveillance
MR campaign	Measles and Rubella campaign
BLAST	Basic Local Alignment Search Tool
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10473-w.

Supplementary Material 1

Supplementary Material 2

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Author contributions
Alireza Esmaeiloghli Amiryli: Methodology, Formal Analysis, Investigation, Writing-Original Draft . Simin Abbasi: Investigation, Formal Analysis . Faezeh Tarpoor: Investigation, Formal Analysis . Azadeh Shadab: Investigation, Formal Analysis . Azam Saboori: Resources . Nazanin-Zahra Shafiei Jandaghi: Investigation . Vahid Salimi: Conceptualization, Writing - Review & Editing, Visualization, Supervision . Talat Mokhtari-Azad: Conceptualization, Writing - Review & Editing, Visualization, Supervision. All authors read and approved the final manuscript.

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Data availability
Data for this study will be available upon request.

Declarations

Ethical approval and consent to participate
This study was carried out and approved by the ethical committee of the Ministry of Health in Iran according to national guidelines and as a part of the national measles surveillance conducted by the National Measles Laboratory (NML) since 2002. Written consent was obtained from all patients or their parents before study enrollment and sample collection. This study was approved by the Review Board of the Tehran University of Medical Sciences, Iran (IR.TUMS.SPH.REC.1401.146).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Conflict of interest
All authors declare that there are no conflicts of interest.

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