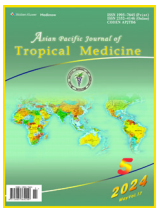

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/apjtm.apjtm_182_24

Resurgence of measles virus infection in an eliminated country, Sri Lanka

Merennage Anusha Yasanthi Fernando[1], Gankandage Prabhath Chathuranga[2], Kaanthaka Gunaradha Abeygunasekara[2], Dedunu Dias Weligamage[3], Janaki Indira Abeynayake[2]✉

[1]*Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Colombo, Sri Lanka*

[2]*Department of Virology, Medical Research Institute, Colombo 08, Sri Lanka*

[3]*Department of Medical Administration, Medical Research Institute, Colombo 08, Sri Lanka*

ABSTRACT

Objective: To describe the situation of measles in Sri Lanka from May to November, 2023 and to define the role of virology laboratory towards case confirmation and epidemiological and genetic characterization of the outbreak.

Methods: This retrospective study analyzed all samples tested for measles from 1st of May to 30th of November, 2023 at National Measles Rubella Laboratory, Sri Lanka. According to the World Health Organization (WHO) algorithm, serum and oropharyngeal/nasopharyngeal swabs were tested with WHO recommended kits for anti-measles IgM and measles virus specific RNA, respectively. Selected RNA positive samples were sequenced at reference laboratory, India. Analysis of sequencing data and construction of phylogenetic tree were carried out at National Measles Rubella Laboratory. Data was analyzed using descriptive statistics.

Results: Of the total 1 132 serum samples and 497 oropharyngeal/nasopharyngeal swabs from 1 326 patients, 657 (49.5%) patients were confirmed as measles by anti-measles IgM, measles virus specific RNA or both. Males (55.6%, n=365) and the age group from >20 to ≤30 years (33.0%, n=217) predominated positive patients. All provinces reported measles positive cases. All samples sequenced (100%, n=42) were genotype D8 with 95.2% (n=40) bearing Victoria.Australia origin.

Conclusions: We described resurgence of measles in an eliminated country, confirming the genotype to be D8, one of the two genotypes currently circulating globally. Further, the study strongly convinced the importance of a strengthened virological surveillance system in an eliminated country, despite its eliminated status.

KEYWORDS: Measles elimination; Resurgence of measles; Sri Lanka; Genotype D8; National Measles Rubella Laboratory

1. Introduction

Measles is a highly contagious, vaccine-preventable disease contributing to a significant public health burden globally, with marked challenges for elimination in all World Health Organization (WHO) regions. It requires high population immunity for interruption of transmission and has been included as a core indicator of impact in the immunization agenda 2030, Measles & Rubella Partnership, to achieve its targets[1]. Moving forward towards elimination, the circulating measles genotypes have been limited to B3 and D8[2,3].

Significance

Following certified measles eliminated in 2019, Sri Lanka reported a case of measles virus infection in May, 2023. Almost half of the patients tested for measles virus infection during the study period were laboratory confirmed as measles. Males and the age group >20 to ≤30 years predominated positive patients. Genotype D8 was the only genotype detected among the sequenced samples with the majority bearing Victoria. Australia origin. The study strongly highlights the importance of a strengthened virological surveillance system in an eliminated country, despite its eliminated status.

✉To whom correspondence may be addressed. E-mail: janakiiabeynayake@yahoo.com; m15591@pgim.cmb.ac.lk

How to cite this article: Fernando MAY, Chathuranga GP, Abeygunasekara KG, Weligamage DD, Abeynayake JI. Resurgence of measles virus infection in an eliminated country, Sri Lanka. Asian Pac J Trop Med 2024; 17(5): 208-213.

Article history: Received 18 March 2024 Revision 24 April 2024
Accepted 10 May 2024 Available online 30 May 2024

Following the last reported case of indigenous measles in May 2016[4,5], Sri Lanka, belonging to the WHO South-east Asia region, was certified measles eliminated in 2019, paved by the strong immunization campaign and the sensitive surveillance system. The country reported a series of cases close to elimination; however, the endogenous transmission was limited to a period less than one year, not interfering with the elimination goals[6]. This was the last reported outbreak of measles in Sri Lanka until May, 2023. Nevertheless, owing to the highly infectious nature of the virus and its circulation in neighboring countries, possibility of imported cases and outbreaks continuously remain a threat to the eliminated status.

In an eliminated setting, a country needs to investigate all suspected cases of measles comprehensively, in an accredited laboratory, utilizing quality-controlled methods. Further, the laboratory should be armed for final case classification and investigation of chains of transmission, with the aid of clinical and epidemiological data[7]. In line with the Global Measles and Rubella Laboratory Network, the WHO accredited National Measles Rubella Laboratory, Sri Lanka carries out this robust laboratory surveillance for measles to promptly detect any importation of cases to the country. Thus, this study aimed to describe the resurgence of measles virus infection in Sri Lanka, from May to November, 2023 and to discuss the role of the virology laboratory towards case confirmation and epidemiological and genetic characterization of the outbreak.

2. Materials and methods

This retrospective study analyzed all the samples of suspected measles virus infection received at the National Measles Rubella Laboratory, Sri Lanka, under the fever-rash surveillance, from 1st of May to 30th of November, 2023. Samples were tested adhering to the measles/rubella testing algorithm updated according to 9th South-east Asia region virologists meeting, September, 2022. All blood/serum samples received were tested for anti-measles IgM, with WHO recommended, commercially validated and in-house verified Euroimmun Anti-Measles virusNP-IgM Enzyme linked immunosorbent assay (ELISA) (Luebeck, Germany).

Oropharyngeal samples received within 7 days of onset of the rash were tested in parallel, for measles virus specific RNA, with measles virus specific real time reverse transcription polymerase reaction (RT-PCR), utilizing WHO recommended and Center for Disease Control (CDC, Atlanta, USA) evaluated primers and probes, along with the superscript-enzyme (Invitrogen SuperScript111 Platinum, USA).

End point RT-PCR was performed on selected measles virus RNA positive samples, with forward and reverse primers of MeV216 and MeV214 respectively, using the Qiagen One step RT-PCR, to generate sequencing templates. The products were sent to the Regional Reference Laboratory, Indian Council of Medical Research-National Institute of Virology, Mumbai, for sequencing. The results of N-450, the 450 nucleotides encoding the 150 amino acids of the carboxyl-terminal of the nucleoprotein, with 12% nucleotide variation between genotypes, were used for genotyping of the virus[8]. Analysis of the raw sequencing file and construction of the phylogenetic tree based on 450-nucleotide sequence of the *N*-gene generated from the samples, in comparison with the reference strain, were carried out at National Measles Rubella Laboratory, utilizing ReCall and MEGA-7 software, respectively. Data was analyzed using descriptive statistics.

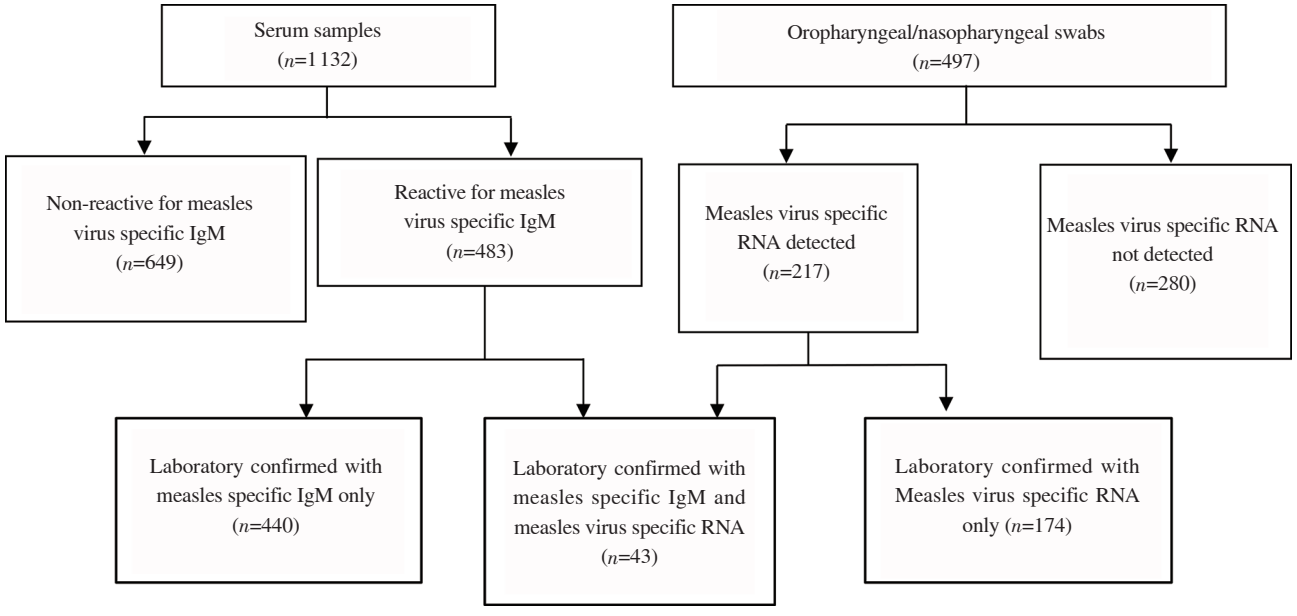

Figure 1. Flowchart of the study.

3. Results

A total of 1132 serum samples and 497 oropharyngeal/ nasopharyngeal swabs were received from 1326 patients suspected with measles virus infection. 657 (49.5%) Patients were positive for measles by anti-measles IgM (67.0%, 440/657), measles virus specific RNA (26.5%, 174/657) or both (6.5%, 43/657) (Figure 1).

Age of the positive cases ranged from one month to 61 years, while majority (33.0%, *n*=217) belonged to the age group from >20 years to ≤30 years (Figure 2). Males (55.6%, *n*=365) predominated positive cases while data on sex was not available in 3.0% (*n*=20) of the patients. All provinces reported measles positive cases during the study period while the highest number was reported from Western Province (68.0%, *n*=447) (Figure 3). Most number of positive cases was reported in the month of August (Figure 4). All measles virus confirmed samples that were sequenced (100%, *n*=42) detected genotype 8. Phylogenetic analysis of sequencing data revealed 95.2% (*n*=40) of these samples to have Victoria. Australia origin while the rest (*n*=2) had Ahmadabad.India as the origin (Figure 5).

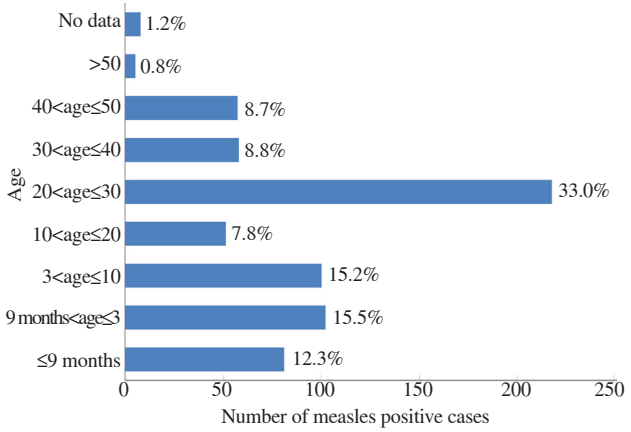

Figure 2. Age distribution of the measles virus positive cases. Age is shown in years unless otherwise specified.

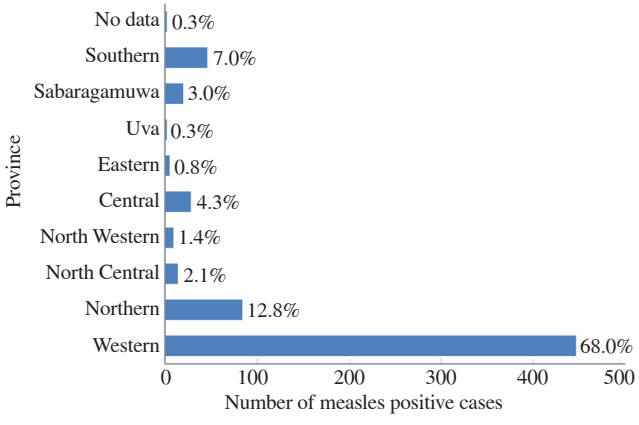

Figure 3. Demographic distribution of the measles virus positive cases.

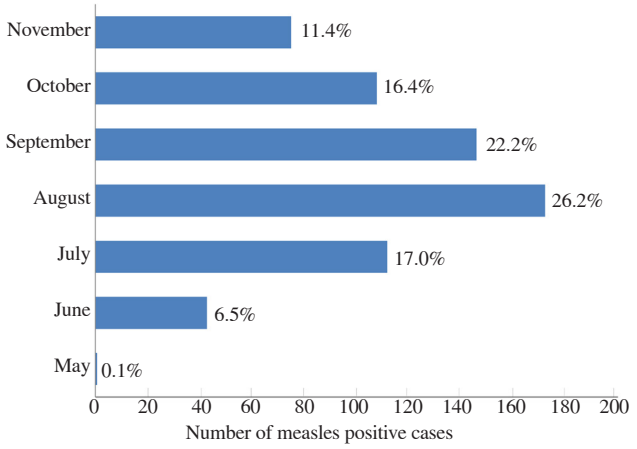

Figure 4. Stratification of measles positive cases during the study period.

4. Discussion

Despite being certified as measles eliminated in 2019, Sri Lanka remains at risk of importation of measles and transmission among under-immunized individuals.

Through the sensitive laboratory surveillance carried out, the first case of measles virus infection was detected during latter part of May, 2023, indicating a measles outbreak in the country. The patient was a 23-year old male from Western Province of Sri Lanka who presented with fever and a maculo-popular rash for 5 days duration.

Of the total samples tested, 49.5% were laboratory confirmed as measles virus infection. Majority of the positive patients were males which was in line with a multi-year pooled analysis showing male predominance in populations less than 45 years of age[9].

The measles vaccination was introduced to the national programme of immunization, Sri Lanka in 1984, as a single dose vaccine given at 9 months of age[10]. The second dose of measles vaccine at 3 years of age in the form of measles-rubella vaccine was introduced in 2001 and two doses of mumps-measles-rubella vaccine has replaced the above two vaccines since 2011[11]. Thus, all individuals less than 40 years of age in Sri Lanka should ideally be vaccinated with one dose of the measles vaccine while all individuals less than 25 years of age should have received both doses. However, the highest percentage of measles cases during the study period was observed in patients with ages between more than 20 years and less than or equal to 30 years of age. Further, patients less than or equal to 40 years of age represented 92.7% (*n*=609) of the positive cases although primary and reinfection were not differentiated among these patients.

12.3% Of the positive samples belonged to infants less than 9 months of age who have not received the measles vaccine, and only had protection from passive transfer of maternal antibodies. It is documented that passive transfer of maternal antibodies against

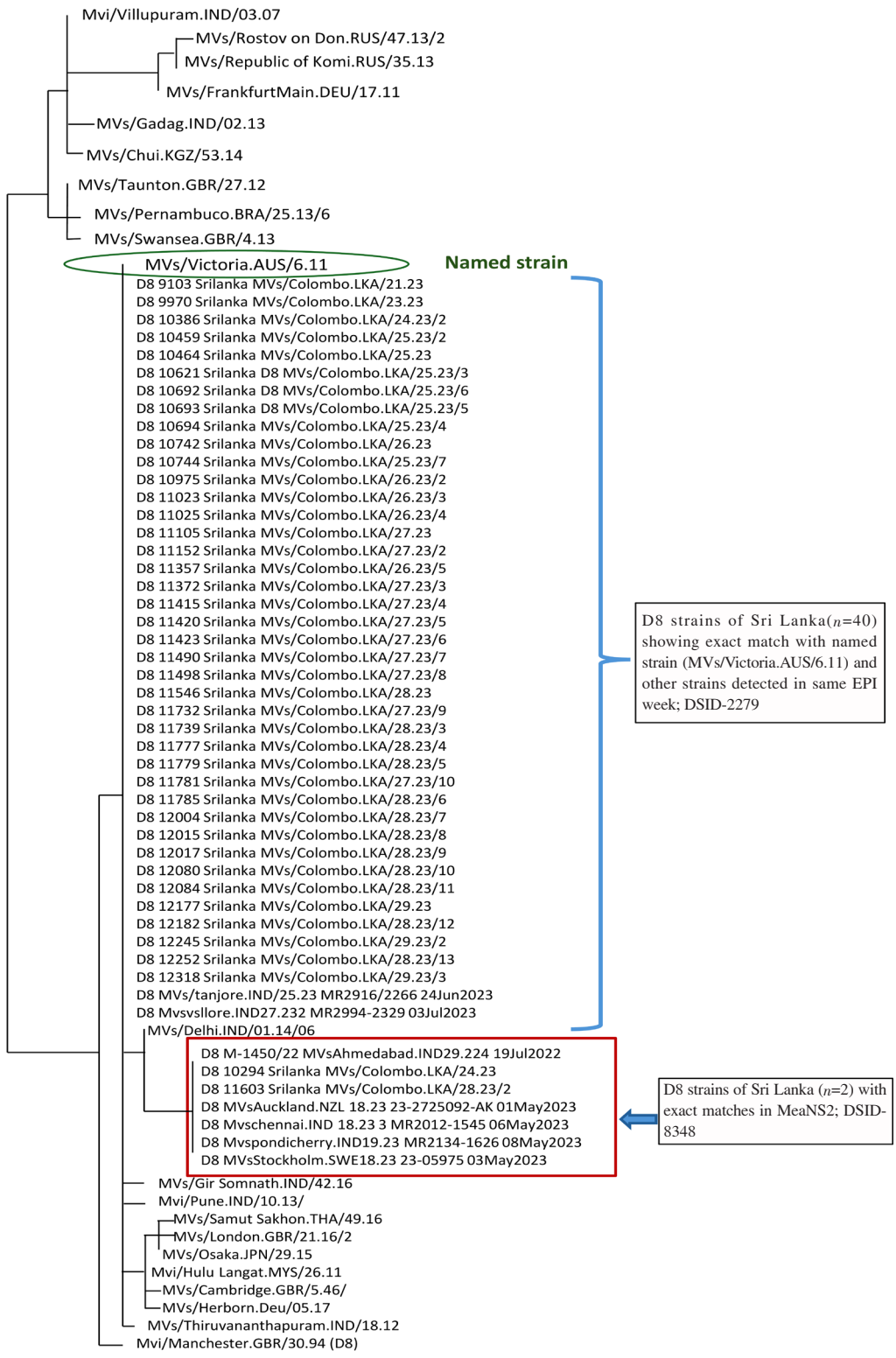

Figure 5. Phylogenetic analysis of 42 measles D8 genotypes detected from Sri Lanka.

measles virus is not sufficient to prevent infection in 92% of the infants by the age of 3 months and 100% by 6 months. Further, the degree of protection offered to infants from vaccinated mothers is limited, which is more common in the current context, compared to mothers following natural infection with the wild virus. Thus, infants are at risk of infection until the first dose of measles vaccination, which was observed during the present outbreak[12,13]. To combat this immunization gap, the Ministry of Health in Sri Lanka has introduced an additional dose of mumps-measles-rubella vaccine to infants between 6 and 9 months of age currently. 27.9% (*n*=183) Of the positive patients belonged to ages less than three years, emphasizing the future challenge to be faced with the possible

devastating complication of subacute sclerosing panencephalitis, which is far more common among patients developing measles during early years of life[14].

Molecular epidemiology of the measles virus is an important component of outbreak investigation and surveillance. Cases were detected in all geographical regions of the country highlighting its spread throughout the country. All samples sequenced detected genotype D8 representing one of the measles virus genotypes in global circulation currently[2]. Measles virus of Victoria.Australia origin predominated the outbreak and has continued to circulate within the country from the first confirmed case to date, endangering the measles eliminated status in Sri Lanka. Transmission of the measles virus of Ahamedabad. India origin was limited among the cases, and continuous surveillance will be necessary to establish or exclude its transmission in the country.

The WHO accredited National Measles Rubella Laboratory, Sri Lanka carries out the robust laboratory surveillance to the country through investigation of all suspected cases with measles presenting with fever and rash, and providing accurate, complete, and timely results for further interventions. The laboratory utilizes updated testing algorithms and diagnostics recommended by the WHO and generates results including genetic characterization of the virus for a complete epidemiological investigation. It follows external quality assurance programmes periodically as per the WHO recommendations, in addition to the internal quality assurance activities carried out, to ensure the quality of the results generated. Thus, the laboratory confirms prompt detection of the measles virus in the country to maintain its eliminated status.

With the high number of sample influx, the utilization of IgG and IgG avidity testing was limited to differentiate primary and reinfection among the positive cases. Moreover, data on vaccination was recruited from the information sheets received with the samples to the laboratory, thus were only available in 1.5% (n=10), limiting its utility for analysis.

In conclusion, we described the resurgence of measles virus infection in an eliminated country, confirming the genotype to be D8, one of the two genotypes currently circulating globally. Further, the study strongly convinced the importance of a strengthened virological surveillance system in an eliminated country, despite its eliminated status.

Conflict of interest statement

All authors would like to declare that there are no potential conflicts of interest.

Acknowledgments

The authors would like to acknowledge the staff of the Department of Virology, Medical Research Institute, Sri Lanka, ICMR-NIV, Mumbai and WHO Regional Coordinator of the South-East Asia Regional Laboratory Network for their support.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

MAYF got involved in designing the study, literature search, statistical analysis and writing of the manuscript. GPC and KGA participated in data acquisition, analysis and the statistical analysis. DDW participated in designing the study as well as in manuscript editing and reviewing. JIA conceptualized and designed the study and participated in manuscript editing and reviewing. The manuscript has been read and approved by all the authors and the requirements for authorship as stated have been met. Each author believes that the manuscript represents honest work.

References

[1] Minta AA, Ferrari M, Antoni S, Portnoy A, Sbarra A, Lambert B, et al. Progress toward measles elimination worldwide, 2000-2022. *MMWR Morb Mortal Wkly Rep* 2023; **72**(46): 1262-1268.

[2] Brown KE, Rota PA, Goodson JL, Williams D, Abernathy E, Takeda M, et al. Genetic characterization of measles and rubella viruses detected through global measles and rubella elimination surveillance, 2016-2018. *MMWR Morb Mortal Wkly Rep* 2019; **68**: 587-591. doi: 10.15585/mmwr.mm6826a3.

[3] World Health Organization. *Measles and rubella global update, distribution of measles genotypes (last 12 months). MeaNS2 database (genotypes) and IVB database (incidence) as of 2023-09-12,WHO immunization data portal.* [Online]. Available from: https://immunizationdata.who.int/assets/measlesrubella/Global_MR_Update_May_2023_SA.pptx. [Accessed on 9 January 2024].

[4] World Health Organisation. *Sri Lanka eliminates measles, News release, India, 9 July 2019.* [Online]. Available from: https://www.who.int/southeastasia/news/detail/09-07-2019-sri-lanka-eliminates-measles#:~:text=New%20Delhi%3A%20The%20World%20Health,globally%20measles%20cases%20are%20increasing. [Accessed

on 20 January 2024].

[5] World Health Organization. *Striving to eliminate measles and rubella in the WHO South-East Asia Region, 3 May 2023*. [Online]. Available from: https://www.who.int/southeastasia/news/feature-stories/detail/striving-to-eliminate-measles-and-rubella-in-the-who-south-east-asia-region#:~:text=The%20challenges%20to%20achieve%20measles,remained%20limited%20in%20endemic%20countries. [Accessed on 10 January 2024].

[6] Samaraweera B, Mahanama A, Ahamad AZ, Wimalaratne GI, Abeynayake J. The laboratory investigation of a measles outbreak in the eve of its elimination in Sri Lanka. *J Clin Virol* 2020; **122**: 104230.

[7] Hübschen JM, Bork SM, Brown KE, Mankertz A, Santibanez S, Mamou MB, et al. Challenges of measles and rubella laboratory diagnostics in the era of elimination. *Clin Microbiol Infect* 2017; **23**(8): 511-515.

[8] Centers for Disease Control and Prevention. *Genetic analysis of measles viruses*. [Online]. Available from: https://www.cdc.gov/measles/lab-tools/genetic-analysis.html#:~:text=Measles%20virus%20genotyping%20can%20play,the%20connection%20is%20not%20obvious. [Accessed on 17 January 2024].

[9] Green MS, Schwartz N, Peer V. Gender differences in measles incidence rates in a multi-year, pooled analysis, based on national data from seven high income countries. *BMC Infect Dis* 2022; **22**(1): 358.

[10] Sri Lanka Medical Association. G*uidelines and information on vaccines*. [Online]. Available from: https://slma.lk/wp-content/uploads/2014/12/Guidelines-and-information-on-vaccines.pdf. [Accessed on 13 January 2024].

[11] Ministry of Health. *Nutrition and indigenous medicine, circular on measles, rubella, and congenital rubella syndrome elimination initiative, Sri Lanka, February, 2017*. [Online]. Available from: https://www.epid.gov.lk/storage/post/pdfs/measles%20_rubella_crs_guidelines_2017.pdf. [Accessed on 13 January 2024].

[12] Hackethal V. *Passive measles immunity wanes quickly in infants, medscape infectious diseases, November 21, 2019*. [Online]. Available from: https://www.medscape.com/viewarticle/921664?form=fpf. [Accessed on 13 January 2024].

[13] Szenborn L, Tischer A, Pejcz J, Rudkowski Z, Wójcik M. Passive acquired immunity against measles in infants born to naturally infected and vaccinated mothers. *Med Sci Monit* 2003; **9**(12): CR541-CR546.

[14] Rocke Z, Belyayeva M. *Subacute sclerosing panencephalitis, StatPearls Publishing; January, 2024*. [Online]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560673/. [Accessed on 28 January 2024].

Publisher's note

Edited by Zhang Q, Pan Y, Lei Y