

## Epidemiology of measles cases and phylogenetic analysis of the virus circulated in the Federation of Bosnia and Herzegovina during 2018: implications for elimination efforts

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### ABSTRACT

**Aim** To present combined measles cases data and phylogenetic analysis of the virus circulated in 2018 in the Federation of Bosnia and Herzegovina (FB&H, the entity of Bosnia and Herzegovina), in order to analyse endemic transmission patterns of circulating strains and its implications for elimination efforts.

**Methods** The data were derived from epidemiological case investigations and laboratory diagnoses based on serology, molecular detection and genotyping of the measles virus.

**Results** During 2018 16 measles cases were reported in FB&H, of which five were classified as laboratory confirmed cases, one was an epidemiologically linked case and 10 were clinically compatible cases. Among them 12 (75.00%) cases were unvaccinated or had unknown vaccination status. The most affected population was up to 14 years of age (13/16; 81.25%). None of the cases was fully vaccinated. Viruses of other genetic lineages had been introduced in FB&H in the recent period. Two virus lineages of genotype B3 were identified. Phylogenetic analysis indicated the presence of a unique sequence of measles B3 virus in FB&H (Sarajevo).

**Conclusion** Further strengthening of measles surveillance system and renewed efforts to increase vaccination levels are necessary to prevent disease and for elimination setting.

**Key words:** genotype, measles virus, phylogeny, vaccination, virus diseases

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## INTRODUCTION

Despite undertaken efforts on the issue of measles and rubella elimination in Europe by 2015, the goal was not achieved. The European Regional Verification Commission for Measles and Rubella Elimination (RVC) concluded that based on data for 2017, 37 (70%) of the Member States provided evidence to demonstrate the elimination of endemic measles (interrupted transmission for at least 36 months) (1). In the WHO European Region, vaccine coverage of  $\geq 95\%$  with two doses of a measles-containing vaccine through high-quality routine immunization services must be achieved and maintained in order to terminate endemic transmission of the measles virus (2). In 2017 approximately 110,000 people died from measles worldwide, mostly children under the age of 5 despite the availability of the safe and effective vaccine (3).

Another important strategy to reach elimination of the virus is strengthening of the surveillance system enabling a prompt detection of suspicious cases and outbreaks (2). Monitoring of the global distribution of viral genotypes has been already recommended by WHO (4). Phylogenetic analysis of the measles virus represents an important asset of its characterization in order to determine pathways of virus distribution in a given geographical area, at a specific time (5). This approach assesses the sensitivity of laboratory capacities for detection and tracking of imported cases. Furthermore, identification of virus genotype in confirmed cases is an indicator of national and global achievements for elimination of measles by documenting the interruption of transmission of endemic viruses (2).

The recent epidemiological situation in the Balkans region and countries across Europe has shown several measles outbreaks (6). Most patients affected by the outbreaks had not been vaccinated (around 95%) and belonged to two age categories, those younger than 5 and over 30 years of age (7).

Bosnia and Herzegovina (B&H) experienced measles outbreaks in the past years (8-10). RVC concluded that measles elimination status in B&H is endemic for 2017 (1).

The aim of this study was to present combined measles cases data and phylogenetic analysis of the virus that circulated in 2018 in the Federation of Bosnia and Herzegovina (FB&H, the entity

of Bosnia and Herzegovina) in order to analyse transmission patterns of the strains and their implications for elimination efforts.

## MATERIALS AND METHODS

### Study design

The research was conducted in the period from 1 January to 31 December 2018 in the Unit for Clinical Microbiology, Clinical Centre of the University of Sarajevo, FB&H. Compulsory epidemiological data were filled out by physicians in the form recommended by the World Health Organization (WHO) for reporting of measles cases. Laboratory investigation was based on blood (serum) samples tested for anti-measles IgM serology. Positive serology results were confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) from nasopharyngeal swabs primarily, or serum samples, if swab was not available. Further analysis involved virus genotyping and phylogenetic characterization in order to identify the transmission route of the virus.

### Methods

**Epidemiological surveillance.** B&H is comprised of two entities, FBiH and the Republic of Srpska (RS) and Brčko District (BD) (a self-governing administrative unit which is under the international supervision). FB&H itself has a federal structure and consists of 10 autonomous cantons. Cantons are then subdivided into municipalities. RS is divided directly into municipalities. A case-based surveillance of measles in FB&H is a passive surveillance system. It relies mainly on health workers who report suspected measles cases to local epidemic services and cantonal public health institutes. Epidemiological investigation of measles cases and obtaining specimens for laboratory testing are not routinely performed. A standard notification form is then sent to the Institute for Public Health of FB&H.

**Serology.** Routine laboratory confirmation of suspected cases was based on the detection of specific anti-measles IgM antibodies using commercial indirect ELISA kits and processed by fully automated instrumentation systems either at the Clinical Centre of the University of Sarajevo, Unit for Clinical Microbiology (Enzygnost Anti-Measles Virus/IgM; BEP 2000 Advance; Sie-

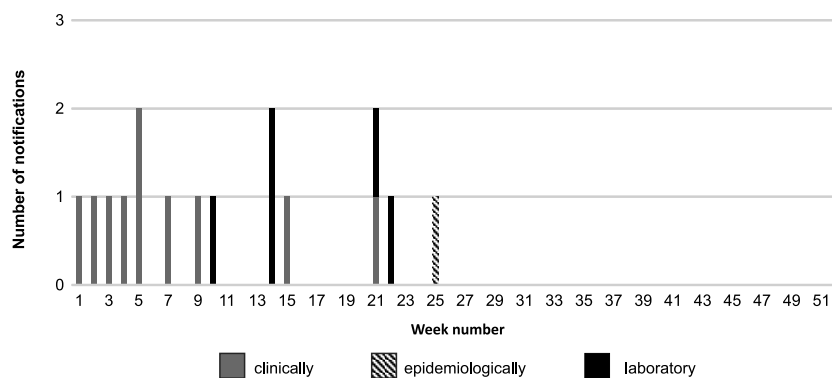


Figure 1. Reported measles cases (n=16) according to the week number, Federation of Bosnia and Herzegovina, 2018.

mens, Marburg, Germany) or at the University Clinical Centre Tuzla, Polyclinic of Laboratory Diagnostics, Department of Microbiology (Anti-Measles Virus IgM Abs.; Alegria; Orgentec Diagnostika, Mainz, Germany), respectively, according to the manufacturer’s instructions.

**Measles virus detection and genotyping.** Real-time RT-PCR detection of viral RNA was done in parallel with serology according to the CDC diagnostic procedure. Positive samples were genotyped using CDC Measles Virus Genotyping kit for RT-PCR and subsequent cycle sequencing. Testing was carried out at the Clinical Centre of the University of Sarajevo, Unit for Clinical Microbiology. Viral RNA extraction from nasopharyngeal swab or serum samples was done according to the QIAamp Viral RNA Mini kit procedure (Qiagen, Hilden, Germany).

**Sequence and phylogenetic analysis.** Sequences were analysed by SeqScape Software v2.5 (Applied Biosystems, USA), BioEdit version 7.0.9.0 (11) and molecular evolutionary genetics analysis (MEGA7) software (12-14). Neighbor-Joining phylogenetic tree based on the Kimura 2-parameter model (14) was constructed using 450 nucleotides coding for the COOH-terminal 150 amino acids of the measles virus nucleoprotein.

**Statistical analysis**

Descriptive statistics were expressed by frequency, sum and percentages.

**RESULTS**

**Epidemiology of measles cases**

In 2018, 19 suspected cases of measles were reported to the FB&H Institute of Public Health. Three cases were discarded after epidemiological investigation or laboratory testing, and 16 cases were classified as follows: five laboratory confirmed cases, one epidemiologically linked case and 10 clinically compatible cases. Cases were reported during the first half of the year (Figure 1) from five of ten FB&H cantons: Una-Sana - two cases, Tuzla - five cases, Central Bosnia - one case, Herzegovina-Neretva - four cases and Sarajevo Canton - three cases.

Reported cases were representative in almost all age groups (Table 1), wherein the most affected population was up to 14 years of age (13/16; 81.25%). Among all cases, twelve (75.00%) were unvaccinated or had unknown vaccination status. None of the cases was fully vaccinated. Four clinically compatible cases were reported as partially vaccinated and further epidemiological investigation was not performed.

Table 1. Age and vaccination status of laboratory confirmed, epidemiologically linked or clinically compatible measles cases, Federation of Bosnia and Herzegovina, 2018

Vaccination status	Number (%) of measles cases in the age group (years)							Total
	< 1	1-4	5-9	10-14	15-19	20-29	≥30	
0 doses	3	3	1	1	0	0	1	9 (56.25)
1 dose	0	0	2	2	0	0	0	4 (25.00)
2 or more doses	0	0	0	0	0	0	0	0
Unknown	0	1	0	0	0	1	1	3 (18.75)
<b>Total</b>	3 (18.75)	4 (25.00)	3 (18.75)	3 (18.75)	0	1 (6.25)	2 (12.50)	16 (100.00)



**Figure 2. Origin of three laboratory confirmed measles cases, Bosnia and Herzegovina (B&H), 2018.** Federation of B&H (FB&H; dark grey), Republic of Srpska (RS; light grey), Brčko District (medium grey) and municipalities with measles cases described (asterisks): Vlasenica (RS; Case 1), Sarajevo (Sarajevo Canton, FB&H; Case 2), Čitluk (Herzegovina-Neretva Canton, FB&H; Case 3).

### Laboratory investigation

In 2018, laboratory testing was done for 27 persons (serology, real-time RT-PCR), resulting in six measles IgM and 4 measles virus RNA positivity. One laboratory confirmed case was not listed among reported cases to the Institute for Public Health of the FB&H, because the patient originated from Vlasenica, Republic of Srpska (RS). Sampling adequacy enabled genotyping of the virus for three of four cases that were analysed (Figures 2,3).

### Case investigation of three laboratory confirmed cases

Measles genotyping was done for three laboratory confirmed measles cases (Figure 2) including one case from Vlasenica, (RS) who was treated in Tuzla (Tuzla Canton, FB&H). For other cases it was not possible to perform genotyping due to inaccessibility of an adequate sample within a recommended time frame or because of patients (parents) refusing a cooperation.

Case 1: One-year old, unvaccinated male from Vlasenica (RS), with manifestation of measles disease was admitted to the Clinic for Children's Diseases of Belgrade, Serbia, then to Zvornik Hospital (RS) and finally to the Clinic for Infectious Diseases, University Clinical Centre of Tuzla

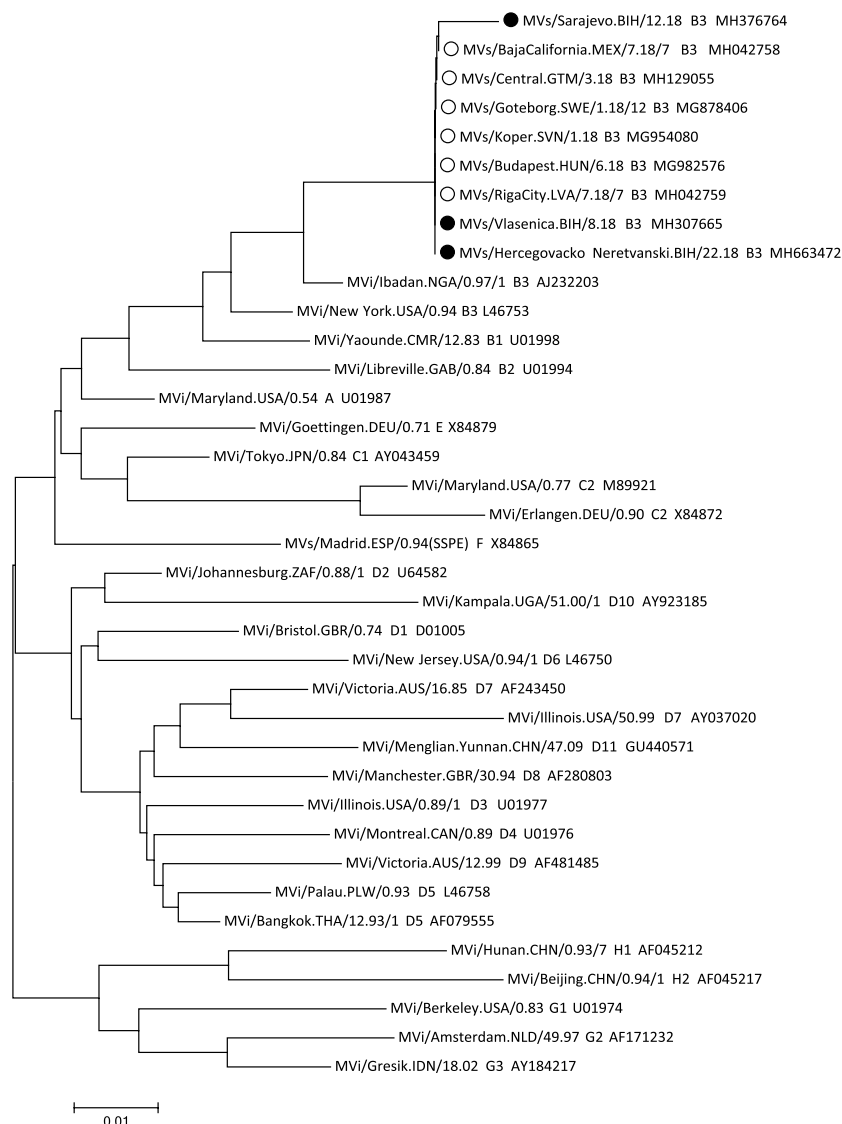
(Tuzla Canton, FB&H). Rash onset was noticed on 25 February 2018 and *pneumonia bilateralis* occurred as a complication of the infection. Blood sample and nasopharyngeal swab were collected on 28 February 2018, and received in laboratory on the same day for virus confirmation.

Case 2: A four-year old, unvaccinated male from Sarajevo (Sarajevo Canton, FB&H) (linked to the outbreak in Kosovo) with the disease manifestation and rash onset on 24 March 2018. The patient was admitted to the Clinic for Infectious Diseases, Clinical Centre of the University of Sarajevo. Blood sample and a nasopharyngeal swab were collected on 29 March 2018 and received in the laboratory on the same day for virus confirmation.

Case 3: A twelve-year old unvaccinated female from Čitluk (Herzegovina-Neretva Canton, FB&H), was linked to her younger brother who had also not been vaccinated and with the disease manifestation (rash onset of her brother was on 19 May 2018; the case was confirmed by serology). The rash onset was observed on 2 June 2018. The patient was admitted to the Clinic for Infectious Diseases, University Clinical Hospital Mostar (Herzegovina-Neretva Canton, FB&H). Blood sample was collected on 7 June 2018 and received in the laboratory on the same day for virus confirmation. The patient was supplementary immunized by MMR vaccine ten days after her brother became ill (on 24 May 2018). Her second younger brother became ill shortly after the disease was confirmed in Case 3, as well as a nurse who provided medical care to her. All cases were symptomatically treated without any complication of the disease.

### Genotyping and phylogenetic analysis

Phylogenetic analysis of the obtained sequences showed that sequence of Sarajevo differs in three nucleotides from those two from Vlasenica and Čitluk (Herzegovina-Neretva Canton). The BLAST fits revealed a 100% identity with measles sequences from Vlasenica (Case 1, MeaNS sequence ID: 131774 MVs/Vlasenica.BiH/8.18, MH307665) and Čitluk (Case 3, MeaNS sequence ID: 133647 MVs/Hercegovacko Neretvanski.BiH/22.18, MH663472), while the variant from Sarajevo (Case 2, MeaNS sequence ID: 132022 MVs/Sarajevo. BiH/12.18, MH376764) was unique according to the currently available sequences in the GenBank database. Phylogenetic analysis showed the closest



**Figure 3. Phylogenetic relationships of taxa- measles virus B3 genotype, Federation of Bosnia and Herzegovina (FB&H) and Republic of Srpska (RS), 2018.** WHO reference strains are non-marked; the closest BLAST fits are marked with white circles and three sequences of B3 described in the study are marked with black circle. Phylogenetic relationships were inferred using the Neighbor-Joining method (13,14). The optimal tree with the sum of branch length = 0.67175768 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (8-y) and are in the units of the number of base substitutions per site. Evolutionary analysis was conducted in MEGA7 (12). The recommended set of reference sequences (15), current sequence variants of genotype B3 and close BLAST fits of sequences from B&H were included in the phylogenetic analysis. Obtained sequences were submitted to MeaNS (16) (sequence ID: 131774 for Case 1- MVs/Vlasenica.BiH/8.18, 132022 for Case 2- MVs/Sarajevo.BiH/12.18 and 133647 for Case 3- MVs/Hercegovoacko Neretvanski. BiH/22.18) and GenBank (Accession numbers: MH307665, MH376764 and MH663472, respectively).

WHO strain defined as MVi/Ibadan.NGA/0.97/1 measles virus genotype B3 (AJ232203) (Figure 3).

### DISCUSSION

The complexity of the administrative organization and organization of the healthcare system in FB&H with high level of decentralization makes it challenging to perform surveillance activities (17). It was shown that healthcare system barriers

had a critical impact on surveillance and response system in other countries (18).

Furthermore, FB&H has registered a downward trend in the coverage of immunization of children with MMR vaccine (19). In 2017 the coverage reached only 63.9% for the first dose of the vaccine. Lower values of the immunization coverage, of the targeted 95%, have been recorded in all cantons in FB&H, which leaves the possibility of



spreading the virus (19). In support of this, none of the reported cases included in our study was fully vaccinated.

Important components of the measles and rubella surveillance system include routine laboratory confirmation of suspected cases and molecular epidemiological data (2). By linking measles molecular surveillance with appropriate epidemiological information, it is possible to gain insight into the global patterns of circulating genotypes and monitor the progress of the elimination program of the endemic virus (5,20).

Other challenges of effective performance of the surveillance system in FB&H include health workers' attitude regarding reporting and high patient demand, lack of public health staff and their regular performance of epidemiological investigation, lack of training programs, limited available resources and poor infrastructure for the collection, storage and shipment of the samples to the referral laboratory, implementation of electronic database and surveillance system. Studies in different settings reported similar results in measles surveillance systems (21-23). Consequently most of the measles cases are categorized as clinically compatible, not laboratory confirmed or epidemiologically linked according to the WHO guidelines (24). Our review of measles surveillance data showed that only 5 cases (31%) were laboratory confirmed. Clinical diagnosis of measles alone in the absence of serological confirmation is not accurate enough for measles elimination. As studies show, the likelihood that a probable case, meeting the clinical case definition, is actually measles is low without other supporting evidence (25).

Efforts should be made to conduct case investigation for suspected cases of measles and to identify contacts.

In comparison with previous molecular epidemiological data, in 2018 we firstly documented the presence of B3 genotype of measles virus in B&H. During the period between February 2014 and April 2015, FB&H was faced with two waves of a large outbreak of measles. The outbreak involved 5103 measles cases recorded in six of ten cantons of FB&H (26) due to low vaccination coverage. During that outbreak, only genotype D8 of measles virus was identified in FB&H unlike in 2007, when genotype D4 was endemic (10). These epidemics have resulted in the formation of a critical

population of actively immunized people, making this area largely safe from developing new epidemics, at least in the short-term period.

Despite a small number of samples that enabled genotyping of the virus, we identified two different lineages circulating in 2018 in FB&H/RS. However, heterogeneity of isolated viruses reflects the diverse geographic origins. In the period January-May 2018 (as of 5 July 2018), within the European Region, countries with the highest number of measles cases, where B3 genotype occurred, were: Romania (B3: 3053; 85.0%), Russian Federation (B3, D8: 1381; 40.0%), Albania (B3: 1046; 49%), Serbia (B3: 938; 61.0%), Italy (B3, D8: 744, 57.0%) and the United Kingdom (B3, D8: 726; 25.0%) (6).

Although the possibility of importation of the virus from the neighboring countries (primarily Serbia, Kosovo) with a widespread outbreak reported in the investigating period exists by the contact of Case 2, an exact sequence match could not be confirmed.

In response to measles outbreaks that were going on in several countries in Europe and the neighbouring countries (6) posing the risk of spreading and maintaining transmission of the disease in sensitive population areas, the Institute for Public Health of FB&H issued a public announcement on the rise in the number of people suffering from measles in the region (27).

A Tailoring Immunization Programmes (TIP) project is underway in FB&H, which explores drivers and barriers to the vaccination and adopts a comprehensive approach to understand factors that relate to individual, social, cultural, institutional and structural factors, in order to create a long-term strategy to increase vaccination uptake and avoid future disease outbreaks (28).

In conclusion, further strengthening of measles surveillance system by laboratory confirmation or epidemiological linking of all suspected cases, expanding molecular surveillance, and renewed efforts to increase vaccination levels are necessary to prevent the disease and for elimination setting. Although there were few measles isolates available in 2018 for sequence analysis, our findings confirmed that viruses from other genetic lineages were introduced in Bosnia and Herzegovina in the period after the outbreaks in 2014 and 2015. None of the viruses isolated in 2018 was a member of the genotype associated with the resurgence.

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## TRANSPARENCY DECLARATION

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