2018/19 INFLUENZA VACCINE EFFECTIVENESS IN ROMANIA IN A SEASON WITH INFLUENZA A SUBTYPES CIRCULATING

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ABSTRACT

The rapid mutations of influenza A virus determine the constant evolution and requires reformulation on the influenza vaccine almost every year. Between November 2018 and April 2019, influenza A(H1N1)pdm09 and A(H3N2) viruses co-circulated in Romania.

The main objectives of the study were to evaluate the activity types of influenza virus and to determine the effectiveness of vaccination against influenza in 2018/19 season in Romania, which is the main goal of the I-MOVE study (Influenza Monitoring of Vaccine Effectiveness).

The study was based on selecting patients with flu symptoms, medically-attended influenza-like illness (ILI). Biological samples (nasopharyngeal swabs) were collected for laboratory tests as: detection, typing and subtyping, antigenic and genetic characterization. The tests were carried out by National Influenza Centre, Cantacuzino National Medico-Military Institute for Research and Development. A total of 284 samples were collected. Influenza type A virus was detected in 109 samples, classified as cases, which included 67 samples of A(H1N1)pdm09 subtype and 42 A(H3N2) subtype. Influenza B was not detected. The negative samples were classified as controls. The vaccine effectiveness (VE) against A(H1N1)pdm09 among all ages was 7% (95% CI: -454 to 84) and against A(H3N2) among all ages was -78% (95% CI: -715 to 61).

The Romanian data contributed to the European I-MOVE multicentre study whose results are provided to the WHO vaccine composition committee. The low sample size and low overall vaccination coverage (4% among controls) limited the precision of our results. Molecular characterizations of influenza viruses can increase the understanding of viral genetic factors, contributing to reduction in vaccine effectiveness.

Keywords: Influenza virus, virology, virus subtype, vaccine effectiveness.

REZUMAT

Evoluția virusurilor gripale din fiecare sezon necesită reformularea compoziției vaccinului aproape în fiecare an. În perioada noiembrie 2018 - aprilie 2019, au fost identificate în România virusuri gripale de tip A, subtipurile H1N1pdm09 și H3N2.

Obiectivele principale ale studiului au fost de a evalua activitatea virusului gripal și a determina eficacitatea vaccinului gripal în România, în perioada sezonului 2018/19, parte a studiului european I-MOVE (Influenza Monitoring of Vaccine Effectiveness).

Studiul s-a bazat pe selectarea pacienților cu afecțiuni clinice compatibile cu gripa (ILI). În total, 284 de probe biologice (tampoane nazofaringiene) au fost recoltate pentru teste de laborator: detectarea, identificarea tipului și a subtipului de virus gripal, caracterizarea antigenică și genetică, teste efectuate în Centrul Național de Gripă, Institutul Național de Cercetare-Dezvoltare Medico-Militară „Cantacuzino". Virusul gripal de tip A a fost detectat în 109 probe, dintre care la 67 de virusuri gripale a fost identificat subtipul H1N1pdm09, iar la 42 subtipul H3N2. Nu a fost detectată prezența virusului gripal de tip B. Probele cu rezultat pozitiv pentru prezența virusului gripal au fost clasificate drept „cazuri", iar cele cu rezultat negativ ca martor ("control"). Eficacitatea vaccinului (VE) pentru A(H1N1) pdm09 la toate vârstele a fost de 7% (95% CI: -454 la 84), iar pentru A(H3N2) a fost de -78% (95% CI: -715 la 61).

Rezultatele obținute au contribuit la studiul european multicentric I-MOVE și la recomandarea compoziției vaccinului gripal de către Organizația Mondială a Sănătății. Numărul redus de probe și acoperirea vaccinală scăzută (4% în rândul „controalelor") au limitat precizia rezultatelor. Caracterizarea genetică a virusurilor gripale identificate poate spori înțelegerea factorilor genetici virali care pot determina reducerea eficacității vaccinului gripal.

Cuvinte-cheie: virus gripal, virologie, subtip viral, eficacitatea vaccinului.

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INTRODUCTION

Influenza is an acute respiratory disease characterized by the sudden onset of high fever, coryza, cough, headache, prostration, malaise, and inflammation of the upper respiratory tree and trachea. Acute symptoms and fever can persist for 7 to 10 days [1]. People of all ages are affected, but in the elderly, in infants, and in people with chronic diseases, influenza is associated with high mortality [2].

Influenza virus, a highly infectious respiratory pathogen, continues to be a significant threat to global public health. Influenza viruses are the only vaccine preventable viruses that undergo frequent genetic and antigenic changes [3]. The main characteristics which contribute to the rapid evolution of these viruses are: large populations, short generation times and high mutation rates. Every mutation helps the virus to evade the host immune system; the vaccine induced immunity is known to last around 6-12 months, perhaps less [4].

Even if the available seasonal influenza vaccines are only moderately effective, the role of vaccination is recognized in reducing the risk of developing the disease and thus the occurrence of its complications. The vaccine effectiveness (VE) may vary from year to year, between vaccine types and brands, between population subgroups (age-groups, risk groups) and differs also for the various influenza type and subtype outcomes measured [5-11]. The influenza vaccine is reformulated each year and annual revaccination is recommended because the VE from previous seasons cannot be considered in subsequent years [12].

Influenza 2018/19 season description

Virological surveillance of influenza activity in primary care is carried out every year between week 40 and week 20 of the following year, and is based on the participation of general practitioners (GP) from the Sentinels network (15 counties and Bucharest – includes at least 192 GPs, coordinated by the National Centre for Surveillance and Control of Communicable Diseases within the National Institute of Public Health (NIPH)).

The influenza activity during the influenza season 2018/19 in Europe was characterized by influenza A(H1N1)pdm09 and A(H3N2) [13]. In Romania was noticed a co-dominance of influenza A(H1N1)pdm09 viruses and, at a lower level, influenza A(H3N2) viruses during the season. The influenza season started in the week (W) 02/2019, when more than 10% of tested samples were positive for influenza and also the first epidemic week (increase by at least 20% the number of patients compared to expected level - the arithmetic mean of the number of reported cases in three previous weeks - and at least 10% of influenza viruses belonging to the same subtype in the isolates of total pathological products tested). High epidemic activity was registered between W03/2019 to W06/2019 (peak W05/2019). In total, 23,727 influenza-like illness (ILI) cases were reported, 2.1 times more than in the previous season (11,527 ILI cases), with the epidemic peak 3 weeks earlier. Within virological surveillance 2,310 confirmed flu cases were recorded nationwide: 1,198 cases with A(H1N1)pdm09, 557 cases with A(H3N2), 550 cases with untyped A, 4 co-infection with A(H1N1)pdm09 and A(H3N2) and 1 case with type B. One hundred ninety-nine deaths were confirmed with influenza (151 cases with A(H1N1)pdm09, 23 cases with A(H3N2), 24 cases with untyped A and 1 co-infection with A(H1N1)pdm09 and A(H3N2) [14].

Vaccination program, coverage and target population

The Romanian Ministry of Health began the vaccination program on September 15th, 2018 [15], managing to vaccinate 1,329,630 persons belonging to target groups until the end of the season [16]. The influenza vaccination program target groups, as recommended by WHO, are: children 6–59 months of age, pregnant women individuals age 6 months - 64 years old with chronic afflictions (pulmonary, cardiovascular, renal, liver, neurological diseases, metabolic disorders, diabetes, obesity, asthma or HIV infection), elderly over 65 years old, healthcare workers and residents of social institutions. Influvac was the vaccine distributed by the

Romanian Ministry of Health to General Practitioners, but other brands (Vaxigrip, Afluria, Fluarix, Optaflu) were available in pharmacies for individual purchase. The vaccine coverage for 2018/19 influenza season in the population targeted by the influenza vaccination campaign was estimated at 6.8% overall and 20.9% among the elderly [16].

Objectives

The objectives of the study were to measure at primary care level, the effectiveness of seasonal influenza vaccine against laboratory confirmed (PCR positive) influenza, in Romania during the season 2018/19 and to provide influenza VE estimates by circulating type/subtype.

ILI case definition

The ECDC ILI case definition was used:

- Sudden onset of symptoms.
- AND at least one of the following four systemic symptoms:
  - fever or feverishness;
  - malaise;
  - headache;
  - myalgia.
- AND at least one of the following three respiratory symptoms:
  - cough;
  - sore throat;
  - shortness of breath.
- AND the absence of other clinical diagnosis [17]

METHODS

The study population was represented by the individuals with no contra-indication for influenza vaccination who consulted a participating GP if they developed ILI swabbed within 7 days of symptoms onset and who had not received antivirals in the 14 days prior to swabbing. We defined a case as a patient who tested positive for influenza virus by real-time reverse-transcription polymerase chain reaction (rtRT-PCR) and a control as a patient with a swab tested negative for influenza virus.

A person vaccinated was defined if the vaccination occurred more than 14 days before disease onset. The history of vaccination included date of administration and where possible the brand name. The cases vaccinated less than 15 days before ILI onset were excluded and the rest of the patients were classified as unvaccinated.

Each patient was evaluated for the presence of any of the medical conditions: insulin-dependent or non-insulin-dependent diabetes, endocrine diseases, cardiovascular diseases, cancer, immunodeficiency and transplant, chronic pulmonary diseases, non-hematologic neoplasia, renal diseases, cirrhosis/chronic liver diseases, obesity. Severity of underlying condition was measured by the number of hospital admissions for these conditions in the 12 months prior to study inclusion. Also, the number of visits at the family physician in the previous 12 months was recorded. Functional status of the ILI patients was established by the GP taking into consideration the need of assistance to ambulate and/or need of bathing, eating or need of assistance for other basic needs. Low functional status was defined as needing help to bathe or to walk. Information regarding smoking was also collected as: never, former smoker (stopped smoking at least one year before inclusion in the study) or current smoker and use of antivirals, type and date of administration.

The association between vaccination status and other characteristics was measured for both case and control groups.

Vaccine effectiveness was computed as VE = (1 – odds ratio)*100. A 95% confidence interval was computed around the point estimate and the vaccine effectiveness was calculated by virus strain.

Analysis was stratified according to: age groups (0-14, 15-59, 60-81), presence or absence of high risk conditions, time (early influenza season, peak, and late influenza season) and target group for vaccination.

Multivariable logistic regression analysis was conducted to control for negative and positive confounding.

Specimen collection, storage, transport

For each patient recruited into the study, a nasal/throat swab specimen was collected

by the participating GP for influenza testing. Specimens were placed at 4°C immediately after collection for ≤3 days and transported from the collection site with a coolant to maintain a refrigerated temperature of approximately 2–8 °C. The GP sent the specimen to the Cantacuzino National Medico-Military Institute for Research and Development using the influenza surveillance form as per routine influenza surveillance. Additional supplementary data were collected using specific form and a consent form was signed by all patients recruited into the study.

Laboratory tests

The viral RNA from clinical specimen was extracted using NucleoSpin RNA Virus kit (Macherey Nagel) according to manufacturer's instructions. The specimens were tested for evidence of influenza viruses (type A and type B) by reverse transcription PCR using real-time RT-PCR (with Invitrogen SuperScript III Platinum one-step qRT-PCR System), a sensitive method of detection. When influenza A viruses were detected, a second rRT-PCR analysis was performed on the HA gene for determination of H1N1 pdm09 or H3N2 subtypes. When influenza B viruses were detected, a second rRT-PCR analysis (SNP genotyping) was performed for lineage determination (Yamagata or Victoria lineages).

Specimens with an influenza crossing threshold value of ≤30 were chosen for further genetic and antigenic characterization.

A subset of positive specimens were inoculated into culture, and analysed for antigenic similarity to the vaccine strain by haemagglutination inhibition assay. Influenza virus isolation on MDCK SIAT-1 was performed, usually after 1-2 passages. The presence of multiplied virus was revealed by haemagglutination with 0.5% Turkey red blood cells (type A, subtype H1N1 pdm09) and 0.75% guinea pig erythrocytes (type A, subtype H3N2).

Genetic characterization was done by sequencing the hemagglutinin (HA or HA1) coding region by Sanger (Applied Biosistem) or the whole genome of influenza viruses by NGS (Illumina MiSeq platform) directly on influenza positive clinical samples or in cell supernatants after virus isolation in MDCK cells (random selection). Clinical samples were used preferentially for sequencing even if they had a lower viral load. The HA sequences were analysed using commercial software as Sequencer 5.4.6 [Sequencher® version 5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA] and DNAStar [SeqMan NGen®. Version 12.0. Madison, WI] and uploaded in Global Initiative on Sharing All Influenza Data (GISAID) database [17]. Maximum-likelihood phylogenies were constructed using MEGA X [18] using Tamura-Nei model [19].

Ethical issues

The data were collected only after the written consent of the patient was obtained and the study had the ethical committee approval to test the sample and use the data (CE312/18.12.2018). The study results were not associated with the name of the patients and were analysed anonymously.

RESULTS

A total of 102 GPs from 13 sentinel and non-sentinel counties accepted to participate in the study in 2018/19 influenza season. Of the 284 patients recruited, 239 met the eligibility criteria (110 cases and 129 controls). The reason for exclusions: 8 cases with antivirals prior to swabbing, 22 did not meet the EU ILI case definition, 5 cases having the onset more than 7 days before swabbing (Tables 1-3).

The proportion of patients with at least one chronic condition was similar between controls and influenza cases (16.5 and 13.2 respectively).

Laboratory results

Among cases (n=110), 67 (60.9%) were positive for A(H1N1)pdm09 and 42 (38.2%) for A(H3N2).

Antigenic characterization

Thirty eight influenza samples were isolated on MDCK SIAT-1 cell line (n=28 A(H1N1)pdm09 and 10 A(H3N2)). The 28 A(H1N1)pdm09 strains were antigenically

Table 1. Number of patients recruited and excluded

Romania, Restriction flow chart	Number of patients recruited
Total data received	284
Onset date or swab date missing	0
Exclusions (contraindications for vaccination, did not wish to participate, antivirals prior to swabbing, etc.)	8
Subtracting persons not meeting the EU ILI case definition	22
Subtracting persons with an interval >7 days between symptoms and swabbing	7
Exclusion of controls before ISO week of first case and after ISO week of last case (onset weeks)	8

Table 2. Total number of cases and controls included

Total number of patients included	239
Number of GPs	81
Number of all flu cases	110
Number of controls	129

characterized by haemagglutination inhibition assay (HAI). The isolated strains were antigenically similar with A/Michigan/45/2015, strain that was component of 2018/19 influenza vaccine formula. No cross reactivity was determined with type A subtype H3 –A/ Singapore/INFIMH-16-0019/2016 by HAI and B/Phuket/3073/2013 (B/Yamagata/16/88 lineage). The 10 A(H3N2) strains were only isolated on MDCK SIAT-1 cell line but were not antigenically characterized.

Genetic characterization

From primary care specimens, only influenza A(H1N1)pdm09 and A(H3N2) sequences were provided as no influenza B viruses were detected. A total of 97 positive specimens were selected for genotyping and 45 influenza viruses had been genetically characterized, 25 (55%) were influenza A(H1N1)pdm09, and 20 (35%) A(H3N2). For the other specimens no sequence could be obtained either due to a low viral load (Ct>29) or the quality of viral RNA.

The HA sequences were compared to those of the vaccine virus and representatives of particular genetic groups/subgroups. All 25 characterized A(H1N1)pdm09 viruses belonged to genetic subgroup 6B.1, represented by A/Michigan/45/2015, which is defined by the amino acid substitutions S84N, S162N (introducing a potential N-linked glycosylation site) and I216T in HA1 with the viruses clustering into a genetic subclade designated as 6B.1A and defined by the HA1 amino acid substitutions S74R, S164T (which alters the glycosylation motif at residues 162 to 164) and I295V, the recommended vaccine component for the northern hemisphere in 2018/19 influenza season (Fig. 1). The substitutions D187Y and T216A were also identified in one or more analyzed sequences. The sequences of HA were uploaded in GISAID database under accession numbers: EPI1343640-42, EPI1365008, EPI1365146-47, EPI1393894, EPI1393896-97, EPI1393902-03, EPI1393906-07, EPI1393909, EPI1393915-16, EPI1393919-20, EPI1444009, EPI1444013-14, EPI1485563-66.

All 20 A(H3N2) viruses were attributed to 3C.2a genetic clade represented by A/ Hong Kong/4801/2014 defined by L3I, N128T (resulting in the gain of a potential glycosylation site), N144S (resulting in the loss of a potential glycosylation site), N145S, F159Y, K160T (resulting in the gain of a potential glycosylation site at residue 158), P198S, F219S, N225D and Q311H in HA1 and D160N in HA2, and 3C.2a1b genetic subclade, represented by A/Alsace/1746/2018 define by K92R and H311Q, 62G and R142G or T128A (resulting in the loss of a glycosylation motif) (Fig. 2). Additionally, other substitutions were observed in one or more analyzed sequences: A106V, K121N and S198P. The sequences of HA were also uploaded in GISAID database under accession numbers: EPI1342651, EPI1343359,

Table 3. Description of influenza cases

		Cases			Controls	
		N	%		N	%
Age groups	0-4	14	12.7		18	14.0
	5-14	30	27.3		34	26.4
	15-64	62	56.4		71	55.0
	65+	4	3.6		6	4.7
	Missing	0			0	
Sex	Male	58	52.7		56	43.4
	Female	52	47.3		73	56.6
	Missing	0			0	
Seasonal flu vaccination: current season (vaccination date not missing)	Yes	6	5.5		5	3.9
	No	104	94.5		124	96.1
	Missing	0			0	
Flu type	Control	-	-		129	100.0
	A(H1N1)pdm09	67	60.9		0	-
	A(H3N2)	42	38.2		0	-
	A untyped	1	0.9		0	-
	B Yamagata	0	0.0		0	-
	B Victoria	0	0.0		0	-
	B unknown	0	0.0		0	-
	pos unknown	0	0.0		0	-
Previous seasonal flu vaccination (last season)	Yes	0	0.0		5	3.9
	No	110	100.0		124	96.1
	Missing	0			0	
Any chronic condition (of all chronic conditions collected that are associated with a recommendation for flu vaccination, including obesity and pregnancy if applicable)	Yes	18	16.5		17	13.2
	No	91	83.5		112	86.8
	Missing	1			0	
Delay between onset of symptoms and swabbing (days)	0	1	0.9		7	5.4
	1	41	37.3		38	29.5
	2	40	36.4		41	31.8
	3	16	14.5		27	20.9
	4	8	7.3		8	6.2
	5	2	1.8		3	2.3
	6	0	0.0		2	1.6
	7	2	1.8		3	2.3
Belongs to target group for vaccination	Yes	20	18.2		28	21.7
	No	90	81.8		101	78.3
	Missing	0			0	
Seasonal vaccination brand (among those vaccinated >14 days before onset)	Not vaccinated	104	94.5		124	96.1
	Influvac	4	3.6		2	1.6
	Vaxigrip	2	1.8		2	1.6
	Vaxigrip Tetra	0	0.0		1	0.8
	Missing vaccination date or status	0			0	

EPI1343367, EPI1343628, EPI1414622, EPI1414690, EPI1414703, EPI1414764, EPI1414787, EPI1414804-05, EPI1414807, EPI1414871, EPI1444028, EPI1485967-72.

Vaccine effectiveness

The 2018/19 VE against any influenza among all ages was -36% (95% CI: -400 to 63) (Table 4). The VE against A(H1N1)pdm09 among all ages was 7% (95% CI: -454 to 84). The VE against A(H3N2) among all ages was -78% (95% CI: -715 to 61).

Table 4. Seasonal influenza vaccine effectiveness against any influenza, influenza A(H1N1)pdm09 and influenza A(H3N2) overall and by age group, influenza season 2018/19 (week 49/2018-week 11/2019), for Romania.

DISCUSSION AND CONCLUSIONS

The course of the 2018/19 season (weeks 40/2018 to 15/2019) was characterized by a later start to the influenza season. End-of-season VE estimates indicate an overall low VE against any influenza among all ages and a higher protection of the vaccine against influenza virus (AH1N1)pdm09 compared to A(H3N2), although precision is low.

The antigenic and molecular characteristics of influenza viruses detected in Romania during the 2018/2019 season are described in the study. The study season was characterized by a dominant spread of A(H1N1)pdm09 viruses and by lower circulation of A(H3N2) viruses. Despite many genetic changes, they remained antigenically closely related to the vaccine virus [21].

The overall low VE against A(H3N2) and the comparatively better VE against A(H1N1) pdm09 are consistent with what was observed in a European interim analysis for this season [5].

The low sample size and low overall vaccination coverage (4% among controls) limited the precision of our results. This study is subject to the usual biases in observational studies. However as it uses the test-negative design, confounding due to health care-seeking behavior may be reduced [22]. Also, participating practitioners swabbed all ILI cases, thus reducing selection bias related to swabbing.

The data from this influenza VE study also contributed to the Romanian influenza surveillance, providing additional information, particularly in regard to genetic characterization. This study is part of the European I-MOVE multicentre study, and all data reported here contributed to measuring a pooled European VE in the 2018/19 influenza season.

Influenza vaccination is the most effective preventive measure against influenza and uptake of the 2019/20 should be promoted. Monitoring vaccine effectiveness remains

Table 4. VE against any influenza among all ages

Influenza type	Population	N	Cases;vacc/ Controls;vacc	Crude VE	CI	Adjusted VE	LL CI
Any influenza	All ages	239	110;6;129;5	-43	-382 to 58	-36	-400 to 63
	15-64 year olds	133	62;4;71;4	-16	-383 to 72	-44	-627 to 71
A(H1N1)pdm09	All ages	179	66;2/113;4	15	-378 to 85	7	-454 to 84
	15-64 year olds	95	33;1/62;3	39	-515 to 94		
A(H3N2)	All ages	171	42;3/129;5	-91	-735 to 56	-78	-715 to 61
	15-64 year olds	99	28;2/71;4	-29	-647 to 78		

[1]Adjusted by age and onset date (both restricted cubic spline with 4 knots), chronic condition and sex

[2]Adjusted by age and onset date (both restricted cubic spline with 4 knots)

[3]Adjusted by age in years and onset date as a restricted cubic spline with 4 knots.

[4]Crude

[5]Adjusted by age in years and onset date as a restricted cubic spline with 3 knots.

[6]Crude

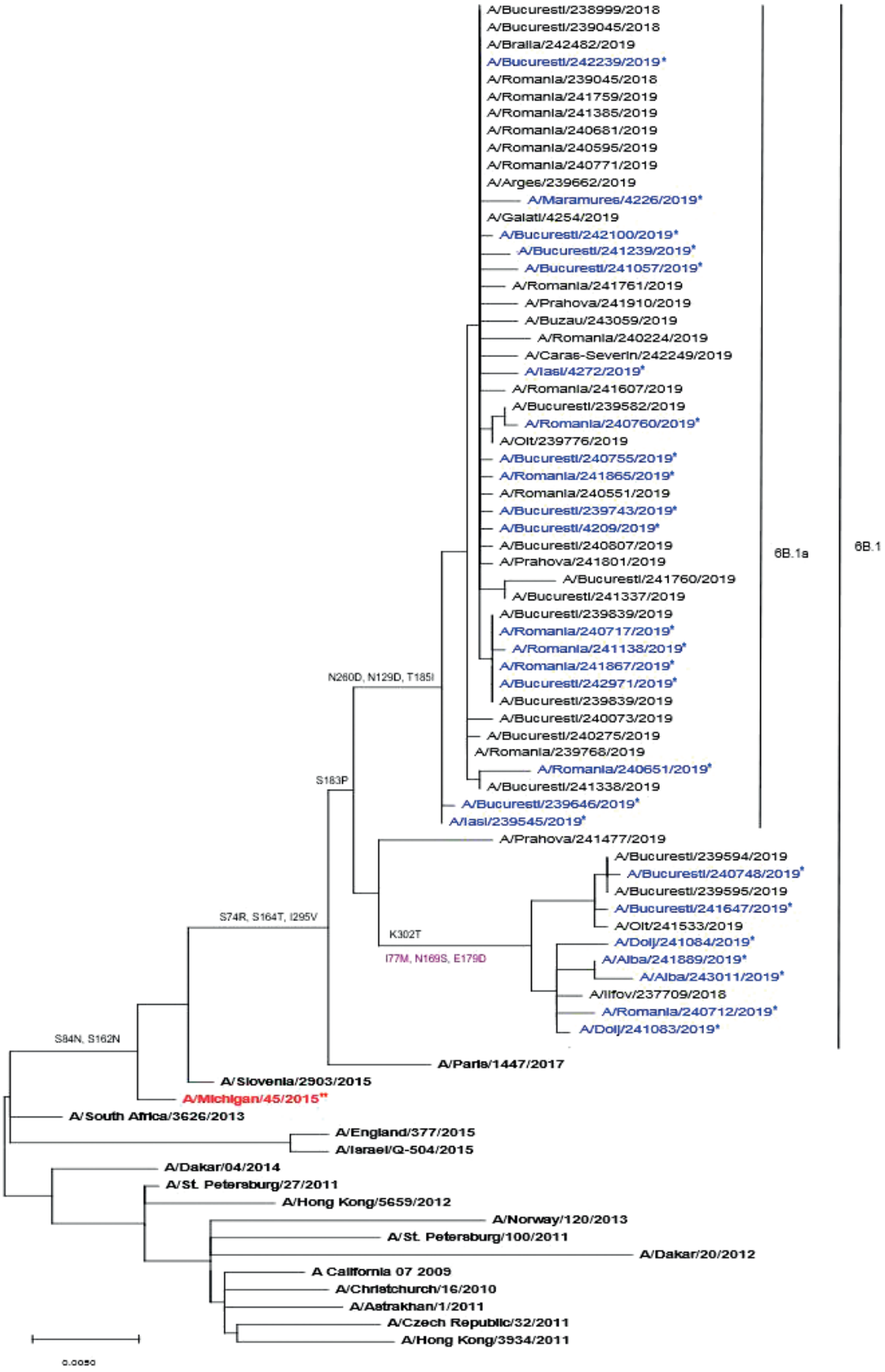

Fig. 1. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes included in the study (*blue) in relation with reference strains (black bolded), vaccine strain (**red) and other Romanian influenza A(H1N1)pdm09 HA strains (Maximum Likelihood, Tamura-Ney model).

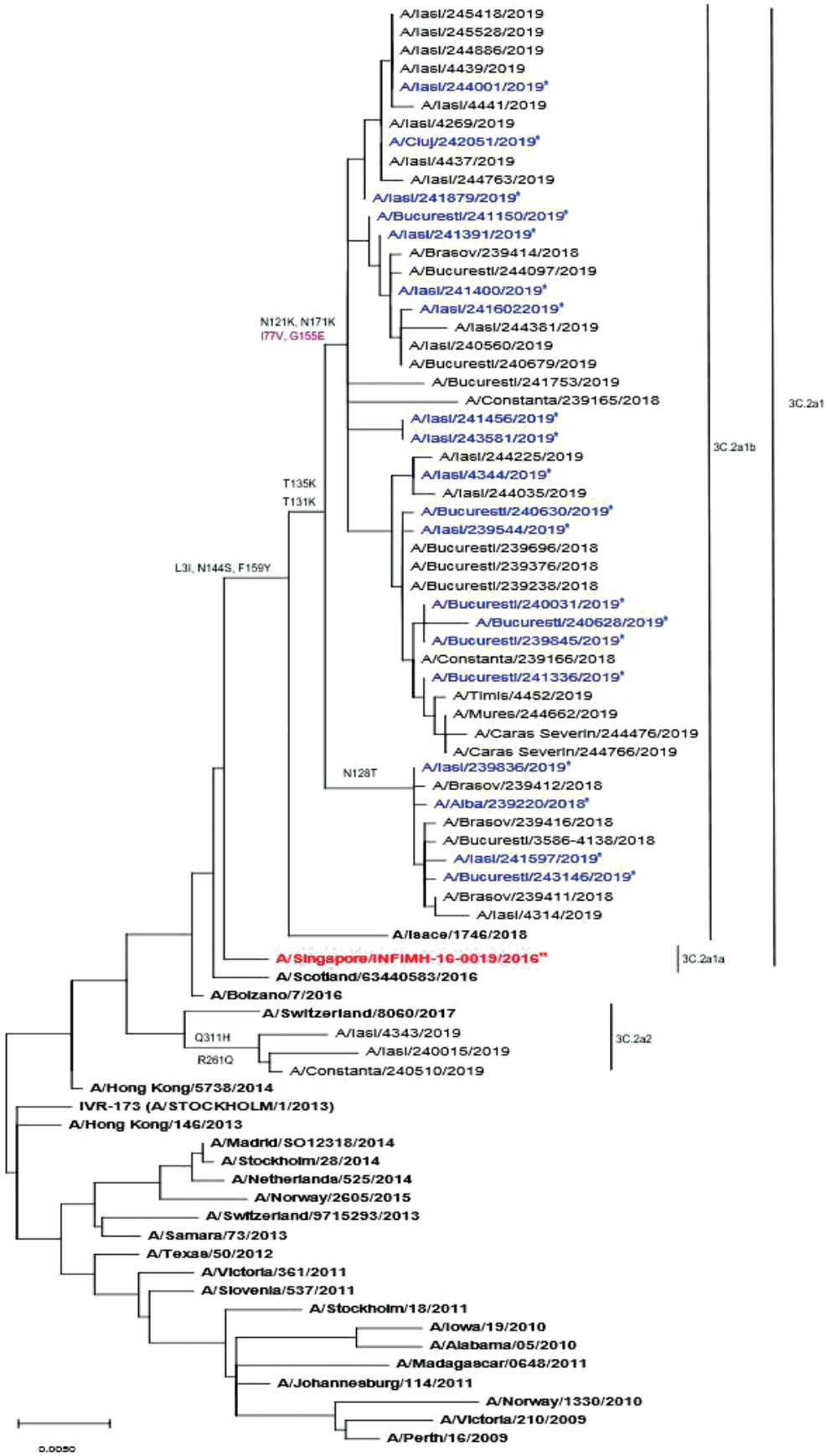

Fig. 2. Phylogenetic comparison of influenza A(H3N2) HA genes included in the study (*blue) in relation with reference strains (black bolded), vaccine strain (**red) and other Romanian influenza A(H3N2) HA strains (Maximum Likelihood, Tamura-Ney model).

important and both a higher vaccination coverage and number of study participants could help increase precision of the VE estimates and thus increase the usefulness of our study.

Conflict of interests: None to declare.

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