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Circulating influenza viruses and the effectiveness of seasonal influenza vaccine in Romania, season 2012-2013

Virusurile gripale circulante și eficacitatea vaccinului gripal sezonier în România, în sezonul 2012-2013

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Abstract

***Backgound**. Using influenza epidemiological and virological surveillance data, we aimed at investigating the profile of influenza viruses circulating in Romania during the season 2012-2013 and estimating the effectiveness (VE) of the seasonal vaccine. **Methods**. We tested all specimens collected from patients with influenza like illness (ILI) in the national surveillance system between week 40/2012 to week 20/2013. Influenza A/B positive specimens identified by molecular detection (RT-PCR) were further characterized. We used hemagglutination inhibition assay for antigenic characterization and chemiluminiscence assay for the antiviral susceptibility testing. Subsequently we conducted nucleotide sequencing of hemagglutinin and neuraminidase genes and phylogenetic tree analyses. We estimated influenza VE using the test negative case-control study design, as 1-odds ratio of vaccination among ILI cases positive for influenza and ILI negative controls. **Results and Discussions**. We tested 1087 specimens, and 537 cases were positive (56.2% influenza B, 40.6% A(H1N1)pdm09, 3.2% A(H3N2). Sixty-four influenza viruses were antigenically and/or genetically characterized. A(H1N1)pdm09 viruses were related to the vaccine strain A/California/07/2009 and clustered with genetic group 6 similar to A/St. Petersburg/27/2011. Influenza B viruses belonged to clade 2 of type B/Yamagata lineage, related to B/Estonia/55669/2011 except one, B/Victoria lineage, representative strain B/Brisbane/60/2008. A(H3) viruses clustered with group 3C of the A/Victoria/208/2009 clade, similar to the vaccine strain A/Victoria/361/2011. All tested strains (57) demonstrated susceptibility to oseltamivir and zanamivir. The adjusted seasonal influenza vaccine effectiveness against influenza A(H1N1)pdm09 (N=119) was 76.9% (95% CI: -113.4, 98.5), suggesting a good protection, consistent with the good match between the vaccine and circulating strains.*

***Keywords**: influenza virus;antigenic/genetic characterization;antiviral susceptibility;vaccine effectiveness.*

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Rezumat

Scopul *studiului a fost de a investiga profilul virusurilor gripale care au circulat în România în sezonul 2012-2013 și de a estima eficacitatea vaccinului gripal sezonier, pe baza datelor de supraveghere epidemiologică si virologică.* ***Metodă.*** *Am testat toate probele colectate din săptămâna 40/2012 până în săptămâna 20/2013, în cadrul sistemului national de supraveghere, de la pacientii cu simptomatologie compatibilă cu gripa. Probele pozitive de gripă A/B identificate prin detecție moleculară (RT-PCR) au fost apoi caracterizate. Am utilizat hemaglutino-inhibarea pentru caracterizare antigenică si chemiluminiscenta pentru testarea sensibilitătii la antivirale. Secventierea genelor codante pentru hemaglutinină si neuraminidază si analiza lor filogenetică a fost de asemenea efectuată. Am estimat eficacitatea vaccinului gripal ca 1-odds ratio folosind un studiu caz-martor cu design negativ.* ***Rezultate și discuții.*** *Am testat 1087 de probe din care 537 au fost pozitive (56.2% gripă B, 40.6% A(H1N1)pdm09, 3.2% A(H3N2). Saizeci si patru dintre acestea au fost caracterizate antigenic si / sau genetic. Virusurile A(H1N1)pdm09 au fost înrudite antigenic cu tulpina vaccinală A/California/07/2009 si au apartinut grupului genetic 6 similar cu A/St. Petersburg/27/2011. Virusurile gripale tip B au apartinut cladei 2 a liniei genetice B/Yamagata, asemănătoare cu B/Estonia/55669/2011, cu excepția unei tulpini care a apartinut liniei B/Victoria, reprezentată de tulpina B/Brisbane/60/2008. Virusurile A(H3) au apartinut grupului genetic 3C al cladei tulpinii A/Victoria/208/2009, asemănătoare cu tulpina vaccinală A/Victoria/361/2011. Toate tulpinile testate (57) au fost sensibile la oseltamivir si zanamivir. Eficacitatea vaccinală ajustată pentru gripa A(H1N1)pdm09 (N=119) a fost de 76.9% (95% CI: -113.4, 98.5), sugerând o protectie bună, în concordantă cu suprapunerea antigenică dintre tulpinile sălbatice circulante si tulpinile incluse în vaccinul recomandat pentru sezonul 2012-2013.*

Cuvinte cheie*: virus gripal, caracterizare antigenică/genetică, suceptibilitate antivirală, eficacitate vaccin*

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Introduction

Influenza is an infectious disease with important public health impact at global level, responsible for pandemics and annual epidemics of different intensity worldwide. Influenza viruses are characterized by genetic and antigenic variability of the surface antigens hemagglutinin (HA) and neuraminidase (NA) – the main targets of the human immune system (1). The most important in public health are influenza types A and B, influenza A presenting different subtypes with subtype A(H3) and A(H1) being the most frequent causes of annual epidemics. Since they emerged in 1968, human influenza A(H3N2) virus strains have undergone a continuous genetic drift, with genetically similar viruses predominating for one or two seasons before receding (2). Replaced in 1957 by subtype A(H2N2), seasonal A(H1N1) strains re-emerged in 1977 developing their own sequential lineage (3, 4). In 2009, a novel and highly transmissible A(H1N1) influenza virus was detected in humans and subsequently caused the last influenza pandemic (5). In addition, two antigenically and genetically distinguishable lineages of influenza B viruses are also currently circulating in humans even though until the ’80s influenza B viruses formed a homogenous group (6, 7).

Emergence and dominance of circulating influenza viruses are variable and difficult to predict, therefore influenza surveillance plays an important role in disease prevention. In Romania, the National Centre for Surveillance and Control of Communicable Diseases (NCSCCD) within National Institute of Public Health (NIPH), Bucharest coordinate the influenza surveillance system. Data is collected by sentinel physicians and NIPH compiles and analyze information on influenza activity at national level and produces the weekly report during the influenza season (8). National Influenza Centre (NIC) within National Institute of Research and Development (NIRDMI) Cantacuzino, part of European Reference Laboratories for Influenza (ERLI Net) of World Health Organization (WHO), has

the main role in monitoring and surveillance of influenza viruses. The NIC provide crucial information necessary to guide designing appropriate vaccines, to plan timing for prophylaxis. It also detects novel/emerging viruses with potential pandemic threat, and assures surveillance of other well-recognized respiratory viruses, important to track seasonality.

Influenza vaccination started in Romania in 1977 to prevent influenza complications in high risk groups. For the season 2012-2013, the trivalent influenza vaccine was recommended to people with chronic diseases (i.e. respiratory, cardiovascular, renal, hepatic diseases, diabetes and metabolic disorders), HIV infected persons, pregnant women, elderly over 65 years old, institutionalized persons for social care and health care workers (8). The influenza vaccination campaign started in early November 2012 and continued throughout the season. The vaccine was provided free of charge for the recommended risk groups, being covered from the Ministry of Health budget. The vaccine was also available in pharmacies. The uptake of seasonal influenza vaccine (provided by MoH) is monitored by NCSCCD.

Since the season 2008-2009, Cantacuzino Institute participates yearly to the I-MOVE network (Influenza Monitoring Vaccine Effectiveness in Europe) (9) with case-control studies aimed to measure the influenza vaccine effectiveness (VE). The studies, conducted in the frame of influenza sentinel surveillance network, followed an adapted generic protocol provided by European Centre for Diseases Surveillance and Control (ECDC) (10).

In the current paper, we aimed to describe the profile of influenza viruses circulating in Romania during the season 2012-2013 taking into account those features involved in guiding of an appropriate annual vaccine composition and to assess the effectiveness of seasonal influenza vaccine.

Materials and methods

During the season 2012-2013, 313 general practitioners (GPs) of the primary health care system distributed across all 41 districts and Bucharest participated in influenza surveillance. They weekly reported the number of patients with influenza like illness (ILI) and acute respiratory infections (ARI) and systematically collected respiratory specimens from ILI cases according to the Romanian methodology and ECDC case definition (11). Collected specimens were sent to NIC, where they were tested for influenza virus.

Virological detection of influenza virus

Viral RNA was purified from clinical specimens or from isolated samples using the NucleoSpin viral RNA virus Kit (Macherey-Nagel Gmbh, Germany). In a first step, we detected the presence of influenza A and B viral genome using real-time reverse transcription one-step polymerase chain reaction (PCR) (Superscript III Platinum One-step qRTPCR System, Invitrogen, Life Technologies, USA).

When influenza A viruses were detected, in a second step, a rRT-PCR analysis was performed on the HA gene for determination of either A(H1N1)pdm09 or A(H3N2) subtypes.

When influenza B viruses were detected, a second one step rRT-PCR analysis was also performed for lineage determination, Yamagata or Victoria-like lineage in a quarter of specimens (12), selected from the first, middle and last period of epidemic.

Characterization of influenza viruses

Phenotypic analysis consisted of hemagglutination inhibition reaction (HI) performed on viral supernatant of Madin-Darby canine kidney (MDCK) cells (ATCC/CCL 34) to evaluate the recognition efficacy of the circulating influenza viruses by the current influenza vaccine-in-

duced antibodies, using reference ferret antisera provided by WHO Collaborating Centres (MRC NIMR, London, UK and CDC Atlanta) and guinea pig (in presence of 20 nM oseltamivir for type A, subtype H3N2) or turkey red blood cells (13).

Genetic characterization was performed by HA gene sequencing of influenza viruses followed by phylogenetic comparison with other known sequences, including those belonging to influenza virus detected in Romania during the previous season.

Antiviral resistance

Phenotypic tests were used to measure the resistance level of influenza virus isolated to neuraminidase inhibitor (NI) antivirals based on enzyme assay with NA-Star 1,2 – dioxetane chemiluminescent substrate (NA-Star Influenza Neuraminidase Inhibitor Resistance Detection Kit) as described elsewhere (14). Briefly, virus dilutions (from cell culture supernatant) were incubated for a short time with neuraminidase inhibitor, and then incubated for 10-30 min with NA-Star substrate. Assay plates were placed in a luminometer (LB 941 multimode reader TriStar – Berthold Technologies). We measured the light signal intensity. We determined the IC50 value of the neuraminidase inhibitor for each viral isolate using a nonlinear curve-fitting, dose-response analysis software (GraphPad Prism™). Viruses were compared to sensitive reference viruses and further characterized by conventional sequencing for identifying specific mutation(s) or novel resistance markers.

Influenza vaccine effectiveness

We conducted a test-negative case control study between week 2/2013 (when first positive case was confirmed in Romania) and week 16/2013 (when the last positive case was recruited in the study). The study followed the same protocol as in our previous published studies (15, 16).

Sentinel GPs from the whole country were invited to participate in the study. Ninety-eight sentinel GPs (31.3%) from 13 districts (31%) accepted and 70 recruited at least one case. The GPs participation was similar as in the last three seasons.

The vaccine effectiveness was computed as VE = 1 – odds ratio (OR) for vaccination among cases (laboratory confirmed ILI patients) versus controls (influenza negative ILI patients). Logistic regression was used to calculate the adjusted OR and its correspondent 95% confidence interval (CI). Variables that changed the crude estimate with more than 10% were included in the model. The statistical analysis was performed with Stata 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

The study was approved by the Cantacuzino Institute ethical committee. No personal data were transmitted with questionnaires at the national level and patients gave a written informed consent to be swabbed (parents or legal tutor in case of children).

Results

Influenza virus detections

In Romania, the influenza 2012-2013 season started in the week 3/2013, when more than 10% of samples tested positive for influenza. Last positive detections were registered during week 16/2013 (Fig 1).

A total of 1087 specimens collected at national level from ILI suspected cases were tested at NIC by rRT-PCR, between week 40/2012 and week 20/2013. Among them, 537 (49.4%) were positive, 302 (56.2%) for influenza B and 235 (43.8%) for influenza A during the influenza activity (week 1/2013 – week 17/2013). Among influenza A viruses, 218 (92.8%) were A(H1N1) pdm09 (including two co-infections A(H1N1) pdm09 and B/Yamagata) and 17 (7.2%) were

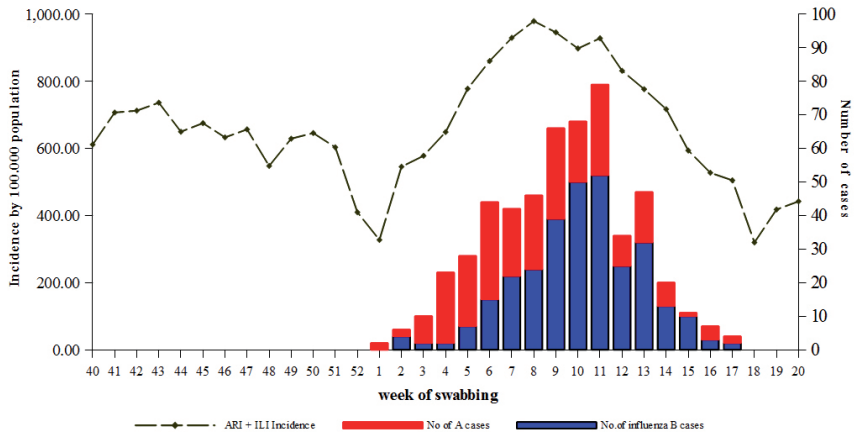

Figure 1. Weekly positive influenza virus molecular detection versus ARI+ILI incidence/100,000 population Romania, season 2012-2013

A(H3N2). Eighty two percent of patients were swabbed within three days of symptoms onset.

Influenza virus characterization

A total of 58 influenza viruses have been isolated from 57 clinical specimens (including one co-infection A(H1N1)pdm09 and B). Among them, 34 (58.6%) were influenza type A (three subtype H3 and 31 pandemic subtype H1) and 24 (41.4%) influenza type B.

Influenza A(H1N1)pdm09 viruses

HI assay suggested that A(H1N1)pdm09 isolates corresponded to viruses recommended by WHO for inclusion in the 2012-2013 Northern hemisphere seasonal influenza vaccine, A/California/7/2009 strain. All 31 strains reacted well with A/California/7/2009 post-infection ferret antiserum (≤2 fold).

The HA genes of the representative A(H1N1) viruses were clustered into eight designated genetic groups, with A/California/7/2009 representing group 1, according to WHO classification (17). Sequencing of eleven A(H1N1)pdm09 strains (including a co-infection H1N1&B – A/Iasi/138230/2013) shown that all viruses fell in group 6, represented by A/St. Petersburg/27/2011, characterized by D97N and S185T substitutions, subgroup 6C carrying additional substitutions (Fig.2).

Influenza A(H3N2) viruses

The A/H3 subtype isolates presented heterogeneity by HI with antiserum against A/Victoria/361/2012 (culture or egg-propagated), or antiserum against the recommended vaccine virus A/Texas/50/2012 (18), particularly for the earliest isolate (week 43/2012), A/Arges/126697/2012. This strain reacted poorly in HI assays (≥8-fold decrease) with postinfection ferret antiserum raised against the previous egg-propagated vaccine virus, A/Victoria/361/2011, compared to the titer of the antiserum with the homologous virus. Nucleotide sequences of HA gene clarified their genetic group. The Romanian strains were characterized by lack of substitutions S45N (gain of a potential glycosylation site) and T48I. All strains had the common HA gene mutations corresponding to group 3C.2, represented by A/Stockholm/18/2011 (Fig. 3). All of the sequences, with one exception (A/Sibiu/132635/2013), carried the substitution T128A (loss of a potential glycosylation site) and R142G.

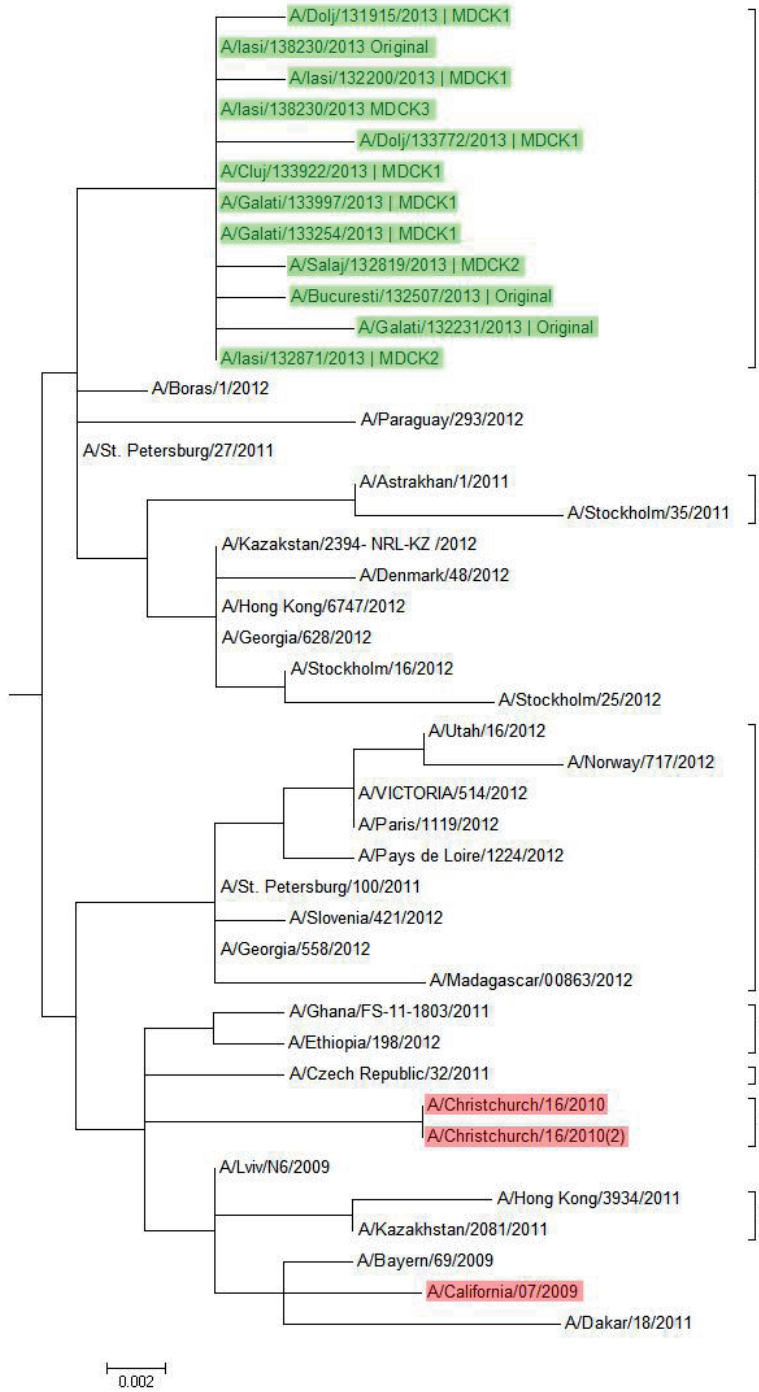

Figure 2. Phylogenetic comparison of A(H1N1) pdm09 HA genes. Highlighted in red vaccine strains and in green strains circulating in season 2012-2013 in Romania All viruses fell in group 6, represented by A/St. Petersburg/27/2011, subgroup 6C carrying additional substitutions: V234I and K283E (e.g. A/Dakar/04/2014), presented in all analyzed sequences. Substitution S185T is missing in the first three isolates of the season (A/Iasi/132200/2013, A/Galati/132231 and A/Dolj/133772/2013).

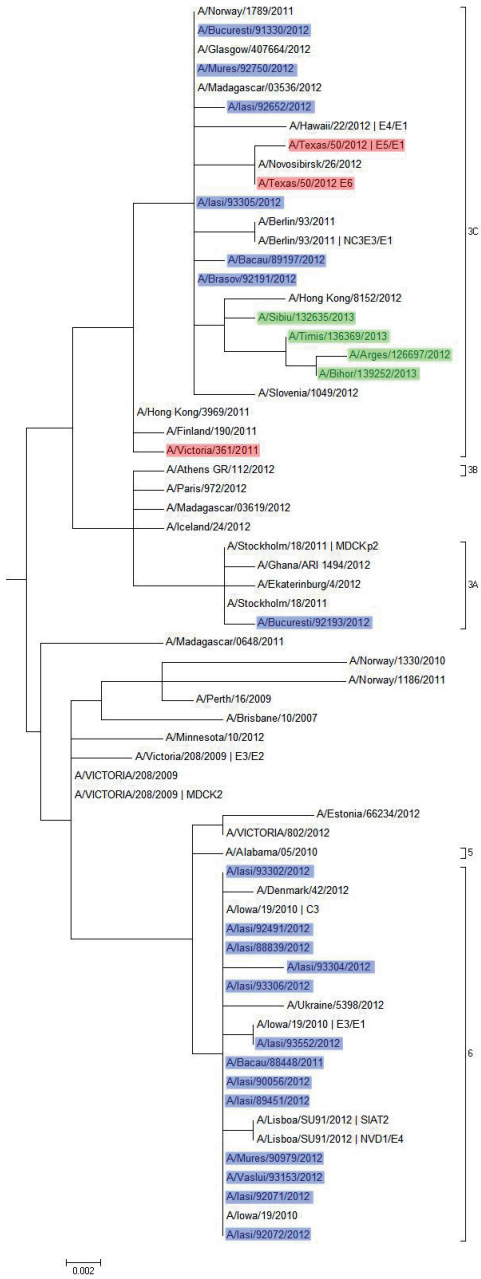

Figure 3. Phylogenetic comparison of A(H3N2) HA genes. Highlighted in red vaccine strains, in green strains circulating in season 2012-2013 in Romania and in blue strains that circulated in the previous season (2011-2012). The 2012-2013 influenza strains had the common HA gene mutations corresponding to group 3C.2 represented by A/ Stockholm/1/2013: Q33R, N145S and N278K, except A/ Arges/126697/2012, having only the first two substitutions.

Influenza B viruses

Of the influenza type B isolates, one isolate (B/Iasi/131732/2013), belongs to B/Victoria/2/87 lineage, confirmed also by genetic analysis, clade 1A, marked by the absence of substitution L58P and few amino acid substitutions compared with B/Brisbane/60/2008.

A total of 23 (95.8%) isolates belonged to B/Yamagata/16/88 lineage, with a good reactivity with vaccine strain B/Wisconsin/1/2010; titers within 4-fold of the homologous titer of the vaccine strain B/Massachusetts/02/2012. The reactivity of the 2012 to 2013 isolates against antiinfluenza virus B/Victoria sera in HI tests ranged between 20 and 40, while the reactivity of the earlier influenza virus B/Yamagata isolates ranged between 160 and 320. However, the HI assay conducted to ambiguous results for 10 strains that were genetically studied for lineage differentiation. All strains, including co-infected B strain, B/Iasi/138230/2013, belonged to line B/Yamagata lineage, clade 2, representative strain B/Estonia/55669/2011, together with the new vaccine strain B/Massachussetts/2/12 (Fig.4). In addition, other 82 clinical specimens positive for type B by rRT-PCR were analyzed by SNP. Among these, all but two were B/Yamagata lineage.

Neuraminidase Inhibitor Susceptibility Assay (oseltamivir and zanamivir)

A total of 57 (10.6%) strains of influenza virus isolated on MDCK were tested in terms of susceptibility to antivirals (oseltamivir and zanamivir) by chemiluminiscence. According to WHO criteria categories (19) for antiviral susceptibility based on IC50 data, all strains had a normal inhibition.

A total of 14 NA genes (10 A(H1N1)pdm09, two A(H3N2) and two influenza B strains) were sequenced for detection of the possible presence of mutations conferring antiviral resistance to neuraminidase inhibitors. None of them dis-

played the presence of known substitutions associated with reduced antiviral susceptibility.

Seasonal influenza vaccine effectiveness

A total of 200 ILI patients were enrolled in the influenza VE study between week 1/2013 and week 17/2013, following the ARI/ILI surveillance evolution at national level (20). After applying the restriction criteria: meeting the EU case definition, symptoms onset starting week 2/2013 (when the first case was confirmed), swabbed within eight days from the onset, confirmed influenza, 197 (98.5%) ILI patients met the inclusion criteria and were analyzed: 130 ILI patients classified as cases and 67 ILI patients as controls. Eight (4.1%) patients were vaccinated, one case and seven controls.

Among cases, 52 (40.0%) were positive for A(H1N1)pdm09, 74 (56.9 %) for B and four (3%) for A(H3N2). There were no significant differences between cases and controls in terms of age, sex, residence (urban/rural), chronic conditions, smoking, functional status and eligibility for vaccination (Table I). The proportion of individuals presenting myalgia was higher among cases than among controls (p=0.004). No other symptom was different between cases and controls. Compared to cases, a higher proportion

Table I. Characteristics of laboratory confirmed medically attended Influenza like illnesses (cases, n=130) and test-negative controls (n=67), Romania, season 2012-2013

Characteristic	Cases n (%)	Controls n (%)	p value
Sudden onset	130 (100)	67 (100)	-
Fever	129 (99.2)	66 (98.5)	0.631
Headache	113 (86.9)	55(82.1)	0.364
Malaise	109 (83.8)	49 (73.1)	0.074
Myalgia	103 (79.2)	40 (59.7)	**0.004**
Cough	125 (96.1)	60 (89.5)	0.066
Sore throat	114 (87.7)	55 (82.1)	0.286
Shortness of breath	39 (30.0)	14 (20.1)	0.172
Mean age (± SD)	32.5 ±19.87	32.7±23.99	0.942
Gender (F/M)	52 (40.0)	26 (38.8)	0.871
Residence (urban/rural)	100 (76.9)	43 (64.2)	0.057
At least one hospitalization in the previous year	5 (3.8)	5 (7.5)	0.273
More than one GP visit in the previous year	24 (18.5)	13 (19.4)	0.873
Any chronic condition	35 (26.9)	21 (31.3)	0.515
Poor functional status	0	3 (4.5)	
Smoking	17 (13.1)	9 (13.4)	0.944
Eligible for vaccination	54 (41.5)	29 (43.3)	0.814
Seasonal vaccination 2012/13	1 (0.8)	7 (10.4)	**0.001**
Pandemic vaccination A(H1N1)pdm2009 in the season 2009/10	6 (4.6)	4 (6.0)	0.682
Any seasonal influenza vaccination in the previous two seasons	7 (5.4)	7 (10.5)	0.190

of controls received seasonal vaccination 2012-2013 (p=0.001). (Table I)

The crude influenza VE against any influenza (N= 197) was 93.3% (95% CI: 45.7; 99.8). The adjusted VE for age, month of swabbing and residence (rural/urban) was 94.5% (95% CI: 43.5; 99.0). The crude VE for patients eligible for vaccination (N= 83) was 94.1% (95% CI: 47.7; 99.9) and the adjusted VE was 97.5% (95% CI: 48.9; 99.8). The adjusted VE against influenza A(H1N1)pdm09 (N=119) was 76.9% (95% CI: -113.4, 98.5).

Discussion

The 2012-2013 influenza season in Romania was unusually long, from the first week of 2013 to week 17/2013, similar to the influenza evolution in European Region, where 2012–2013 influenza season started around week 48/2012, peaking around week 5/2013 and lasting until week 16/2013 (21, 22).

Influenza activity was lower during all the 2012-2013 season, compared to the last two seasons (20) and the season was characterized by a mixed virological picture. A co-circulation of influenza virus type A(H1N1)pdm09 and B/Yamagata lineage viruses was recorded during the whole season, with a slight dominance of type B. These virological findings were not similar to what was observed across the whole WHO Euro region, where influenza type A viruses predominated over type B (62% vs. 38% detections) (22).

The circulation of A(H3N2) was rare compared with subtype A(H1N1)pdm09, and much lower than the European average (34% versus 66%). In HI assay, all viruses reacted well with antiserum against the vaccine strain.

The A(H1N1)pdm09 isolates matched well with the viruses recommended by WHO for inclusion in the seasonal influenza vaccine for the 2012-2013 season in the Northern hemisphere (18). All, except one strain (A/Iasi/132200/2013), carried at least one amino-acid substitution in addition to the three that define the genetic group (D97N, V234I and K283E) but they did not carry additional substitutions that have been documented (H138R with V249L in HA1, subgroup 6A, e.g. A/Hong Kong/5659/2012 or N38D with V173I and N260T). The D222G mutation associated with increased pathogenicity (23) was absent in all tested influenza strains.

The genetic diversity of virus A(H3N2) was limited compared with the previous season (2011-2012). The four viruses that have been genetically analyzed were quite homogeneous, both fell in group 3C, in contrast to previous season (2011-2012) where three different A(H3N2) clades were detected in Romania, when group 6 was predominant (representative A/Iowa/19/2010) but also with limited activity of group 3A and significant group 3C.

Influenza B viruses of the B/Yamagata lineage dominated the 2012-2013 season and fell in clade 2 of HA genes and within the B/Massachusetts/02/2012 clade. They could be differentiated antigenically from those carrying clade 3 HA genes, represented by B/Wisconsin/1/2010. However, not all antisera were able to antigenically differentiate between viruses falling in the alternate clades.

In Romania, none of NA gene sequenced revealed the presence of substitutions associated with reduced antiviral susceptibility. This is consistent to the WHO report that revealed at global level only very low numbers of oseltamivir and zanamivir resistant viruses detected during the season (22). In UK, four unlinked sporadic influenza A(H1N1)pdm09 viruses have been found to be resistant to oseltamivir, two following treatment with oseltamivir and two from patients without any treatment history (24).

The results of the VE case-control study suggested a good protection of the 2012-2013 seasonal vaccine, consistent with the good match between the vaccination and circulating

A(H1N1)pdm09 strain. The VE estimates for any influenza and for A(H1N1)pdm09 were higher than reported by other studies in Europe for that season (25). We can relate this findings either to a better match between the vaccine and circulating strains in Romania or to some limitations of our study that led to an overestimate of the VE results. These limitations are detailed as follows. First, the low number of GPs participating in the study, covering only 13/42 districts might not be representative for the entire country. These are experienced GPs that have participated in the sentinel influenza surveillance for many years, and therefore they could recognize an influenza case among ILI patients based on symptoms only and also recommend vaccination more than other physicians. However this potential selection bias was minimized by the recommendation in the protocol to select all ILI cases attended during the season. Secondly, the small sample size and the low vaccination coverage did not allow measuring the influenza VE with precision or to perform more extensive analyses. The influenza vaccine uptake decreased yearly (from 16.6 % in general population and 49.4% in elderly in 2007/2008 (15) to 4.2% and 14.9%, respectively in 2012-2013 (20). However, the vaccination coverage of controls in our study is higher than the general population one (10% vs 4.2%). We explain this by the fact that population given raise to cases in our study is not the general population but the catchment area of the participating GPs, which for sentinel GPs is higher, also discussed in other studies (26). On the other hand, GPs might be tempted to swab more vaccinated ILI patients to identify vaccine failures. If the controls were more likely to be swabbed if they were vaccinated, the VE would have been underestimated. This bias was minimized by the negative test design itself, as GPs did not know at the moment when recommended swabbing if the ILI patient enrolled will be positive for influenza (i.e. case) or negative (i.e. control).

Conclusion

Our results show through a wide range of laboratory tests, that influenza viruses circulating in Romania in 2012-2013 season were closely related to the vaccine strains and were susceptible to antivirals. The laboratory results are consistent to the influenza VE study which shows a good protection of the vaccine, taken into account the various limitations described above. Influenza surveillance should be sustained and be reinforced by additional health promotion and awareness campaigns that will reestablish the public confidence in influenza vaccination as the main preventive measure against a disease that can be responsible for high morbidity and mortality in future seasons.

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List of abbreviation

NCSCCD - National Centre for Surveillance and Control of Communicable Diseases
NIPH - National Institute of Public Health

NIC - National Influenza Centre
NIRDMI Cantacuzino - National Institute of Research- Development for Microbiology and Immunology Cantacuzino
ERLI Net - European Reference Laboratories for Influenza
WHO - World Health Organisation
ILI - Influenza Like Illness
HA - Hemagglutinin
NA - Neuraminidase
rRT-PCR - Real-time reverse transcription one-step polymerase chain reaction
HI - Hemagglutination Inhibition
I-MOVE network - Influenza Monitoring Vaccine Effectiveness in Europe
ECDC - European Centre for Diseases Surveillance and Control
VE - vaccine effectiveness
ARI - Acute Respiratory Infections
GPs - general practitioners
CDC - Centers for Disease Control and Prevention, Atlanta, USA
NI - Neuraminidase Inhibitor

References

1. Nicholson KG, Wood JM, Zambon M. Influenza. Lancet. 2003 Nov 22;362(9397):1733-45. DOI: 10.1016/S0140-6736(03)14854-4
2. Hay AJ, Gregory V, Douglas AR, Lin YP. The evolution of human influenza viruses. Philos Trans R Soc Lond B Biol Sci. 2001 Dec 29;356(1416):1861-70. DOI: 10.1098/rstb.2001.0999
3. World Health Organization. Influenza. Wkly Epidemiol Rec. 1978;53:21–4.
4. Nakajima K, Desselberger U, Palese P. Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. Nature. 1978;274:334–9. DOI: 10.1038/274334a0
5. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009 Jun 18;360(25):2605-15. DOI: 10.1056/NEJMoa0903810
6. Kanegae Y, Sugita S, Endo A, Ishida M, Senya S, Osako K, et al. Evolutionary pattern of the hemagglutinin gene of influenza B viruses isolated in Japan: cocirculating lineages in the same epidemic season. J Virol. 1990;64:2860–5.
7. Rota PA, Hemphill ML, Whistler T, Regnery HL, Kendal AP. Antigenic and genetic characterization of the haemagglutinins of recent cocirculating strains of influenza B virus. J Gen Virol 1992;73:2737-42. DOI: 10.1099/0022-1317-73-10-2737
8. National Institute of Public Health, Romania. Influenza, ARI and SARI surveillance methodology, 2012-2013. [Metodologia de supraveghere a gripei, infectiilor respiratorii acute si SARI pentru sezonul 2012-2013]. Available on: http://www.insp.gov.ro/cnscbt/index.php?option=com_docman&task=cat_view&gid=41&Itemid=10 (accessed on 7/01/2014).
9. Valenciano M, Ciancio Bc, I-MOVE study team. I-MOVE: a European network to measure the effectiveness of influenza vaccines. Euro Surveill. 2012;17(39):11-21.
10. European Centre for Disease Prevention and Control (ECDC), Protocol for case-control studies to measure pandemic and seasonal influenza vaccine effectiveness in the European Union and European Economic Area Member States, Technical document. Available from: http://www.ecdc.europa.eu/en/publications/Publications/0907_TED_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Case_Control_Studies.pdf
11. European Commission. Commission Decision 2009/363/EC of 30 April 2009 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council, OJ L 110, 1.5.208, p. 58. Available on line:http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:110:0058:0059:EN:PDF
12. Biere B, Bauer B, Schweiger B. Differentiation of Influenza B Virus Lineages Yamagata and Victoria by Real-Time PCR. J Clin Microbiol. 2010;48 (4):1425–7. DOI: 10.1128/JCM.02116-09
13. World Health Organization. Indentification of the haemagglutinin subtype of viral isolates by haemagglutination inhibition testing. Manual for the laboratory diagnosis and virological surveillance of influenza, WHO Press. Geneva. 2011;43-59.

14. Necula G, Mihai ME, Sbarcea CE, Dinu S, Lupulescu E, Ceausu E, et al. Overview of influenza virus antiviral resistance in Romania in the last four epidemic seasons – phenotyping, genotyping and molecular analysis study. Rev Romana Med Lab. 2011;19(3):227-40.
15. Pitigoi D, Lupulescu E, Alexandrescu V, Baetel AE, Sbarcea C, Ionita E, et al. The effectiveness of seasonal influenza vaccine in persons older than 65 years in Romania. Case-control pilot study I-MOVE, 2008-2009, [Eficacitatea vaccinului gripal sezonier la persoanele cu varsta peste 65 de ani din Romania. Studiul pilot caz-martor I-MOVE, 2008-2009]. Bacteriol Virusol Parazitol Epidemiol. 2009 aprilie-iun;54(2):59-67.
16. Pitigoi D, Ivanciuc AE, Necula G, Lupulescu E, Alexandrescu V, Savulescu C. Influenza vaccine effectiveness to prevent medically attended laboratory confirmed influenza during season 2010-2011 in Romania: a case control study. Rev Romana Med Lab. 2012;20(2):127-34.
17. World Health Organization Influenza Centre London. Report prepared for the WHO annual consultation on the composition of influenza vaccine for the Southern Hemisphere 2014, 23rd – 25th September 2013.
18. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2012-2013 northern hemisphere influenza season. Geneva World Health Organisation http://www.who.int/influenza/vaccines/virus/recommendations/2012_13_north/en/ (accessed on 7/01/2014).
19. World Health Organization. Meetings of the WHO working group on surveillance of influenza antiviral susceptibility. Wkly Epidemiol Rec. 2012 Sep 28;87(39):369-74.
20. Pistol A, Popescu R. ARI and ILI surveillance in Romania, week 40/2012-week 20/2013 [Supravegherea infectiilor respiratorii acute si a gripei in Romania, S 40/2012-S20/2013]. Available at: http://www.insp.gov.ro/cnscbt/index.php?option=com_docman&Itemid=49 (accessed on 7/01/2014).
21. European Centre for Disease Prevention and Control (ECDC). Weekly influenza surveillance overview, April 2013. http://www.ecdc.europa.eu/en/publications/Publications/influenza-weekly-surveillance-overview-26-apr-2013.pdf
22. World Health Organization. Summary of the 2012-2013 influenza season in the WHO European Region. 2013 http://www.euro.who.int/__data/assets/pdf_file/0011/195824/Summary-of-the-20122013-influenza-season-in-the-WHO-European-Region-final.pdf
23. Rykkvin R, Kilander A, Dudman SG, Hungnes O. Within-patient emergence of the influenza A(H1N1)pdm09 HA1 222G variant and clear association with severe disease, Norway. Euro Surveill. 2013 Jan 17;18(3). pii: 20369.
24. Public Health England. Surveillance of influenza and other respiratory viruses, including novel respiratory viruses, in the United Kingdom: Winter 2012/13. Available at: http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317139321787 (accessed on 7 January 2014).
25. Kissling E, Valenciano M, Buchholz U, Larrauri A, Cohen JM, Nunes B, et al. Influenza vaccine effectiveness estimates in Europe in a season with three influenza type/subtypes circulating: the I-MOVE multicentre case–control study, influenza season 2012/13. Euro Surveill. 2014;19(6):pii=20701.
26. Savulescu C, Valenciano M, de Mateo S, Larrauri A; cycEVA Study Team. Estimating the influenza vaccine effectiveness in elderly on a yearly basis using the Spanish influenza surveillance network--pilot case-control studies using different control groups, 2008-2009 season, Spain. Vaccine. 2010;28(16):2903-7. DOI: 10.1016/j.vaccine.2010.01.054