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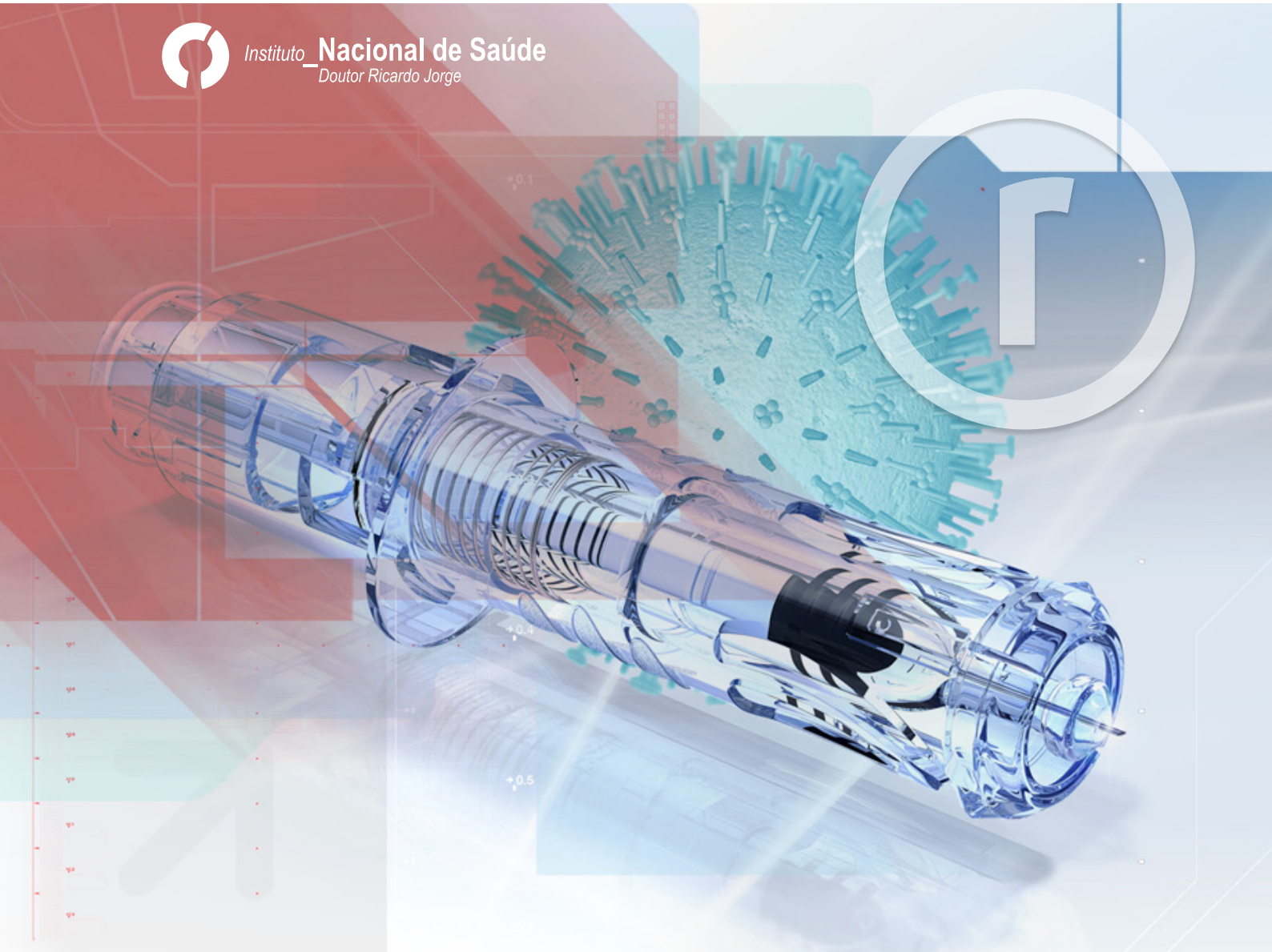
Doutor Ricardo Jorge



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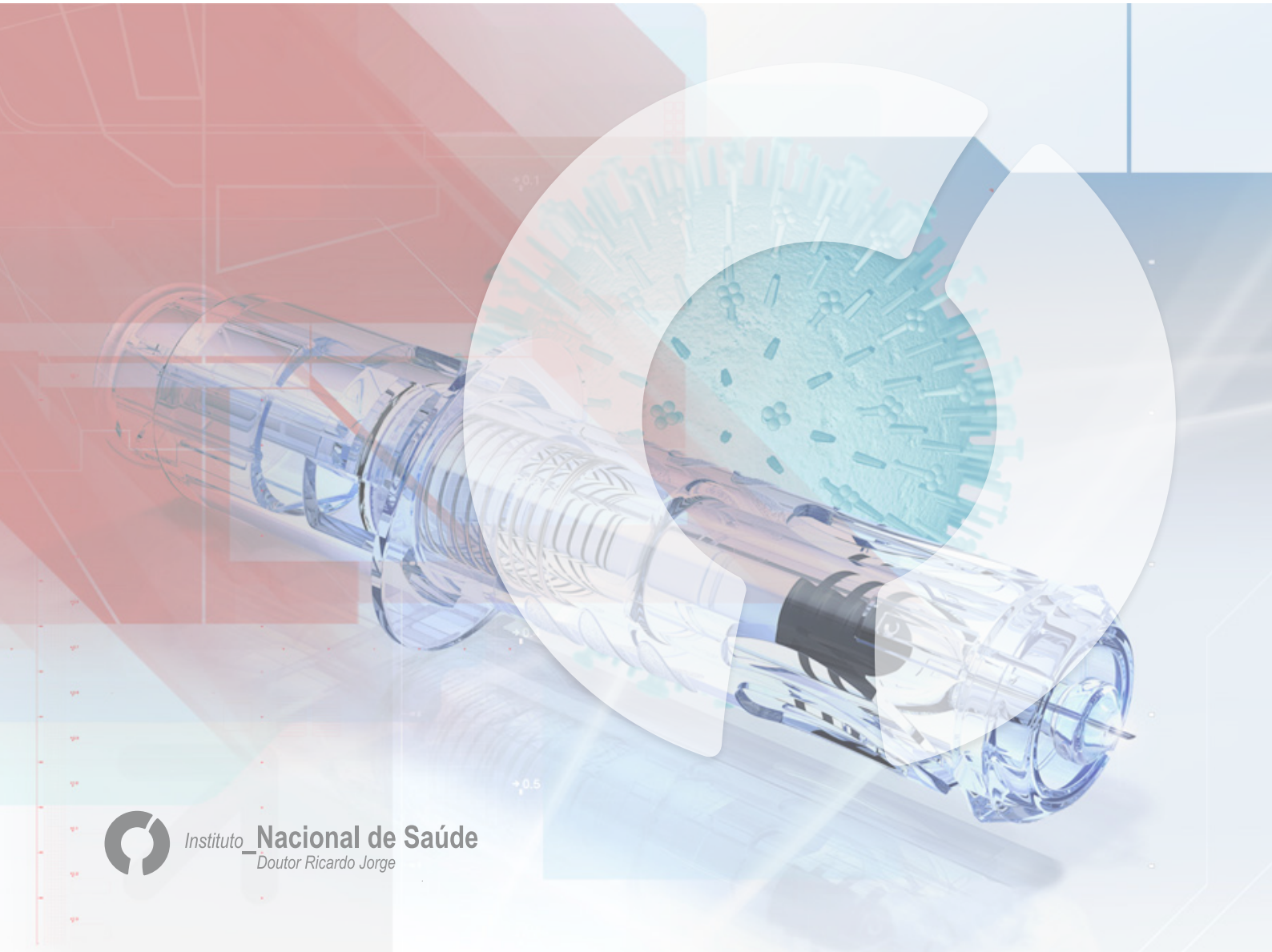

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This report was prepared as part of the Project “Monitoring Influenza vaccine effectiveness during influenza seasons and pandemics in the European Union”, financed by the European Centre for Disease Prevention and Control, and describes the results obtained in Portugal under the Protocol Agreement celebrated between EpiConcept SARL, Paris and National Health Institute Doutor Ricardo Jorge, Lisbon, signed on December 2014.

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SUMMARY

Background

The EuroEVA project is the Portuguese component of the multicentre I-MOVE study. The results to be presented are related to the 7th EuroEVA season and aimed the estimation of 2014-15 end of season influenza vaccine effectiveness in i) all age groups; ii) by risk group; iii) by influenza subtype and thus contribute to monitor VE estimates every year.

Material and methods

The "Protocol for case-control studies to measure seasonal influenza vaccine effectiveness in the European Union and European Economic Area Member States- Portuguese site study version" was implemented entirely with no changes to be added. VE was estimated as one minus the odds ratio of being vaccinated in cases versus controls adjusted for confounders by logistic regression. Potential confounders were investigated and included if they changed crude OR estimate in at least 10% after adjustment by the Mantel-Haenszel method.

Results

In Portugal, influenza epidemic occurred between week 1/2015 and week 8/2015 and had a medium-high intensity. Both B and A(H3) virus were circulating, but with dominance of the first one. A(H1)pdm09 was only detected sporadically.

From the 50 GP's that accepted to participate in the study, 31 GP's effectively participated in the study by selecting patients (which corresponds to a 62% participation rate). A total of 268 ILI patients were enrolled, each GP recruited in average 8.6 patients. After excluding 19 ILI patients the final sample for analyses consisted on 249 ILI patients (147 cases and 102 controls). From the cases, 68% were positive for type B virus, Yamagata lineage, 31% were positive for influenza A (H3) and 1% for A(H1)pdm09. Antigenic and genetic analysis indicated that influenza A(H3) viruses were genetically and antigenically different from the 2014/2015 vaccine strain, most of them belonging to the new virus cluster 3C.2a. Detected influenza B viruses belong to the same lineage (Yamagata) of the strain represented in the 2014/2015 influenza vaccine. Comparing cases and controls, we verified that they were statistically different in relation to:

- **Age:** controls were older than cases (median age in controls was 51 yrs vs. 44 yrs in cases);
- **Any chronic disease:** the prevalence of at least one chronic condition relevant for influenza vaccination was higher in controls (44.1 % vs 28.1%);
- **Seasonal vaccine in 2013-14:** controls were more often vaccinated against influenza in the last season than cases (28.4% vs. 12.3%);
- **Help bathing:** controls needed more help for bathing than cases (0% in cases vs 4.1% in controls).

Overall results indicated that vaccine coverage (VC) in controls was statistically higher ($p<0.001$) than in cases (VC controls=30.7% and VC cases=11.0%). Similar results were obtained for the target group for vaccination by the National Health Authorities (VC cases=26.8% and VC controls=54.9%, $p=0.003$). Restricting the analysis to type B virus (Yamagata lineage), VC was statistically ($p=0.001$) higher in controls than in cases (30.7% vs 8.1% in all ILI cases and 53.9% vs 20.0% in the target group).

After adjustment for age, chronic disease and month of illness onset, VE adjusted estimates in all population was 63.4% (95% CI: 16.2%; 84.0%). In the target group for vaccination, after adjustment, VE estimates was 65.0% (95% CI: 8.1%; 86.7%). Restricting to B Yamagata cases, similar VE estimates were obtained for all population and target group: $VE_{\text{all population}}$ = 78.5% (95% CI: 39.5; 92.4); $VE_{\text{target group}}$ = 79.6% (95CI: 34.7; 93.6). Although all crude and adjusted VE estimates were statistically significant, low precision was observed.

Conclusions

The 2014-2015 season adjusted VE estimates against B/Yamagata was approximately 79% (statistically significant but low precision). No VE estimates against A(H3) were obtained due to small sample size. All influenza B belonged to genetic group 3, different from genetic group 2, represented by B/Massachusets/02/2012 vaccine strain but antigenically these strains are related. This fact is in line with a moderate/high VE for influenza B in 2014-15 season.

RESUMO

O projeto EuroEVA (Efetividade da vacina antigripal na Europa) é a componente portuguesa do estudo europeu multicêntrico I-MOVE. Os resultados apresentados correspondem à implementação do estudo EuroEVA na época 2014-2015 e pretende obter estimativas da efetividade da vacina (EV) sazonal 2014-2015 i) na população geral; ii) no grupo alvo para o qual a vacina é recomendada; iii) por tipo/sub tipo de vírus e desta forma contribuir para a monitorização da EV antigripal todas as épocas.

Material e Métodos

O "Protocol for case-control studies to measure seasonal influenza vaccine effectiveness in the European Union and European Economic Area Member States- Portuguese site study version" foi implemetado na integra sem alterações a reportar. A efetividade da vacina (EV) foi estimada através de EV=1-OR sendo OR o odd ratio de estar vacinado nos casos vs controlos ajustado fatores de confundimento foram analisados e incluídos se alteraram o odds-ratio bruto em pelo menos 10% após ajustamento pelo método de Mantel Haenszel.

Resultados

Em Portugal o período epidémico ocorreu entre as semanas 1/2015 e 8/2015 tendo-se verificado uma atividade gripal média-alta. Verificou-se a co circulação de vírus do tipo B e do sub-tipo A(H3), tendo o primeiro dominado durante a época. Verificou-se ainda a circulação esporádica de vírus do tipo A(H1)pdm09.

De entre os 50 médicos de família (MF) que aceitaram participar no estudo, 31 reportaram doentes com síndroma gripal (SG), correspondendo a uma taxa de participação de 62%. No total foram selecionados 268 doentes com SG, em média cada médico recrutou 8,6 doentes. Após exclusão de 19, por não respeitarem a definição de caso de SG, a amostra final para análise consistia em 249 doentes com SG (147 casos positivos e 102 controlos negativos). De entre os casos, 68% eram do tipo B vírus, linhagem Yamagata, 31% eram positivos para o vírus do subtipo A (H3) e 1% para o tipo A(H1)pdm09. A análise genética e antigénica dos vírus influenza A(H3) revelou que na sua maioria pertencem ao novo grupo genético 3C.2a, que se distinguem da estirpe A(H3) contemplada na vacina da época 2014/2015. Os vírus influenza B detetados pertencem à linhagem Yamagata, à semelhança do vírus do tipo B contemplado na vacina da época 2014/2015. Comparando casos e controlos verifica-se que os grupos eram estatisticamente diferentes nas seguintes variáveis:

- Idade: os controlos eram mais velhos que os casos (idade mediana nos controlos o era 51 anos vs 44 anos nos casos);
- Doença crónica: a prevalência de pelo menos 1 doença crónica com relevância para a infeção por gripe era mais elevada do que controlos (44,1 % vs 28,1%);
- Toma da vacina na época anterior: os controlos tinham uma história passada de toma da vacina (época 2013/14) mais frequente (28.4% vs. 12.3%);

- Incapacidade (Ajuda na toma de banho): os controlos manifestaram em maior percentagem necessidade de ajuda para tomar banho (0% nos casos vs 4.1% nos controlos)

De uma forma geral, a cobertura da vacina (CV) sazonal antigripal 2014/15 foi mais elevada (e com significado estatístico, p<0.001) nos controlos (30,7%) do que nos casos (CV=11,0%). Restringindo a análise para o grupo alvo para a toma da vacina de acordo com as Autoridades de Saúde Nacionais, verificaram-se resultados semelhantes (CV casos=26,8% and CV controlos=54,9%, p=0,003).

Relativamente à análise por tipo de vírus dominante (vírus do tipo B/ Yamagata), a CV era estatisticamente superior (p=0,001) nos controlos do que nos casos (30,7% vs 8,1% na população geral e 53,9% vs 20,0% no grupo alvo da vacina).

Após ajustamento para idade, presença de doença crónica e mês de início de sintomas, a EV ajustada para a população em geral foi de 63,4% (IC95%: 16,2%-84,0%). No grupo alvo da vacina a estimativa ajustada situa a EV em 65,0% (IC95%: 8,1%-86,7%). Restringindo a análise aos casos B/ Yamagata, obtiveram-se valores similares na população em geral e no grupo alvo da vacina: $EV_{\text{população geral}}$ = 78,5% (IC95%: 39,5%-92,4%); $EV_{\text{grupo alvo}}$ = 79.6% (IC95%: 34,7%-93,6%). Embora ambas as estimativas tenham significado estatístico, a precisão é baixa.

Background

Every year the influenza vaccine is reformulated, so estimating the influenza vaccine effectiveness (VE) every season in an early stage is of major importance to support public health decisions. In this context, the National Health Institute Doutor Ricardo Jorge (INSA) in Portugal, conducted during the 2005/2006 and 2006/2007 seasons, two pilot studies with a cohort design[1]. They were designed to provide data from sources independent of health services in order to allow the feasibility of the real study if and when hospitals, health centres, physicians and other health services collapse during a pandemic. Main conclusions drawn from these two pilot-studies stressed that estimation of effectiveness of anti-flu vaccine should be based on multicentre studies involving several European countries.

In the 2008/2009 season, the European Centre for Disease Prevention and Control (ECDC) launched a call for tender directed towards testing several designs in order to select the more appropriate to estimate in-season effectiveness of antiflu vaccine both in seasonal and pandemic influenza. The INSA, through both its Departments of Epidemiology and Infectious Diseases, was included in the project *Monitoring influenza vaccine effectiveness during influenza seasons and pandemics in the European Union* (I-MOVE), by implementing the EuroEVA (Efetividade da Vacina Antigripal na Europa). It consisted in a pilot study conducted to test a study case-control design able to measure in-season and end of season influenza vaccine effectiveness, during the autumn and winter 2008-2009, among people aged 65 years and above, using several control groups[2]. The study was designed to use preferably routine data provided by the Portuguese system of integrated clinical and virology influenza surveillance, based on the General Practitioner (GP) Sentinel Network - Médicos Sentinela (MS). The main results of this pilot study was that the test negative design was suitable for measuring influenza VE and reinforced the need of a multicentric approach so to increase sample size and provide more precise estimates[3].

Since the 2009/2010 season, due to the pandemic, the study population started to include all individuals but with special interest in the target group for vaccination. In Portugal, this group is defined by the General Directorate for Health (GDH) and has included individuals with chronic conditions, health professionals and care providers, pregnant in 2nd and 3rd trimester and elderly with 65 a more years of age. Since 2011-2012, the recommendation for taking the vaccine was extended to the 60-64 years old. The EuroEVA project has adapted the protocol in accordance with the "Protocol for case-control studies to measure seasonal influenza vaccine effectiveness in the European Union and European Economic Area Member States" and the recommendations from the GDH. This season the case-control test negative design was implemented once again and aimed at estimating influenza vaccine effectiveness in i) all age groups; ii) by risk group and iii) by influenza type/subtype.

Materials and methods

Study design

Case-control test negative design (Test Negative Design) where case laboratory confirmed influenza cases (ILI+) were compared to laboratory influenza-negative ILI patients (ILI-).

Study population

The study population was the community-dwelling all aged individuals with no contra-indication for influenza vaccination residents (permanent or visitors) in the catchment area of the participating General Practitioners (GP).

Study period

In order to estimate seasonal VE ILI cases from all ages was selected by GPs starting on final of November 2014 (week 48/2014) and finished in middle of April 2015 (week 16/2015).

The peak of the epidemic was determined through the Portuguese routine surveillance system, "Médicos-Sentinela"[4]. The influenza vaccination campaign started in week 39/2014.

Outcome

The outcome of interest was laboratory-confirmed influenza, by type and sub type.

ILI cases

ILI DEFINITION

A case of influenza like illness (ILI) was defined as an individual who consults a participating GP, presenting ILI signs and symptoms (using EU ILI case definition[5]).

INFLUENZA CASE

An influenza case was defined as an ILI case with a respiratory sample positive for influenza during the study period.

Laboratory analysis

Specimens were collected from ILI cases who consult GP within seven days of symptom onset. The specimens were analysed at the National Influenza Reference Laboratory of the National Institute of Health Doutor Ricardo Jorge.

SPECIMEN COLLECTION, STORAGE AND TRANSPORT

Nasopharyngeal swabs were collected into a suitable transport medium and sent to the laboratory. This procedure was conducted by the GP himself or by a nurse under his supervision.

The specimens on viral transport medium were kept at 0 to 4ºC and within 24 hours were transferred from the GP to the National Influenza Reference Laboratory by an express mail company, following the procedure already in place with the samples collected for routine surveillance of seasonal influenza.

LABORATORY TESTS, STRAIN CHARACTERISATION AND QUALITY ASSURANCE

Each sample was identified anonymously with the ILI case code, and the information related

to the patient, demographic data, characteristics of the disease and the data concerning the confounding variables were recorded on the notification form.

Laboratory confirmation of influenza infection was done using cell-tissue culture for influenza viruses and a real-time multiplex RT-PCR.

Virus isolation is a very useful technique for the diagnosis of influenza infection allowing for further antigenic and genetic characterization of isolates, and also for vaccine preparation or drug-susceptibility testing. Isolates were characterized antigenically by haemagglutination inhibition tests (HAI), carried out using antisera and reference virus strains distributed by WHO Collaborating Center (London) [6]. Selected isolates were sent to the WHO Collaborating Center in London for further study.

The rapid detection and typing and subtyping of seasonal influenza viruses was performed by a multiplex "in house" real-time RT-PCR targeted to the matrix/hemagglutinin and nucleoprotein genes of influenza A and B, respectively. In order to identify the influenza B lineage (Yamagata/88 and Victoria/87), a multiplex "in house" real-time RT-PCR was used.

Influenza virus isolation was performed using MDCK-siat1 cell line, allowing further antigenic characterization of isolates and drug susceptibility assays [6] .

For the influenza virus isolation, 50% of the ILI influenza positives by PCR, from period of recruitment, were inoculated in MDCK siat1 cell culture. The isolated strains were further antigenically characterized.

The phylogenetic analysis of the influenza virus isolates was performed by sequencing the coding region of the HA1 subunit of the hemagglutinin, for a subset of isolates from the beginning, peak and end of the season, using the ClustalW Method for the multiple alignment and the maximum Likelihood Method for the construction of the phylogenetic trees (MEGA Software).

The reference laboratory follows internal control procedures and external quality control programs organised by European Influenza Surveillance Network (EISN) and the World Health Organization (WHO).

Case finding

CASE IDENTIFICATION

Cases were identified among patients that present with ILI to a participating GP. All participating GPs worked in a Health Center of National Health Service (Ministry of Health) and had a stable list of patients. The participating GPs covered all Mainland Portugal and islands, as perfectly as possible. GPs recruited ILI cases from all ages (belonging or not to their patient list), provided that an encounter patient/GP takes place.

A systematic sampling method was used for the recruitment of patients with less than 60 years of age, patients with 60 or more years of age were all included in the study.

The systematic sampling procedure applied to patients <60 yrs, consists on the selection, by each GP, of the first two ILI cases of each week. In order to avoid biases regarding the weekday the first day of the week for each GP was ran-

domly assign (e.g. for GP1 the "week" starts at Thursday, GP2 Tuesday, GP3 Monday, etc.). Each GP received a SMS reminder on the day before the start of his "week".

CASE INCLUSION AND EXCLUSION CRITERIA

Cases were eligible if they meet the above case definition and accept to participate. Written informed consent was collected by the GP.

Cases were excluded if they refuse to participate in the study; are not eligible for influenza vaccination due to a condition listed in the summary of product characteristics; are institutionalised; are unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons. Reasons for exclusion were documented in an appropriate form.

Controls

ILI cases that tested negative for any influenza virus [A(H1N1),A(H3N2) and B] were included in control group.

Test-negative controls were excluded, and reasons were documented, if they refuse to participate in the study; were not eligible for influenza vaccination due to a condition listed in the summary of product characteristics; are institutionalised; were unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons.

Exposure (vaccination)

DEFINITION OF VACCINATION STATUS

An individual was considered as vaccinated against influenza if the vaccination occurred more than 14 days before ILI onset. Seasonal vaccines available in Portugal, in 2014, include: Fluarix, GlaxoSmithKline (non adjuvant); Influvac, Solvay Farma (non adjuvant); Istivac, Sanofi Pasteur MSD (non adjuvant) and Istivac Infantil, Sanofi Pasteur MSD (non adjuvant) – children with 6 to 35 months of age;

VACCINATION STATUS ASCERTAINMENT

Inoculation with 2014/2015 WHO approved seasonal influenza vaccine was ascertained by the GPs:

1. Consulting the patient record and confirming explicitly with the patient if the vaccine was taken;
2. If no data exists in the clinical record, the ILI cases will be asked about vaccine inoculation status.

RISK GROUPS

Individuals were considered to belong to a risk group if in the GP records include or if the patient reports suffering from one of the underlying conditions included in the interview questionnaire.

CONFOUNDING FACTORS AND EFFECT MODIFIERS

The following variables were collected as they could be confounders between the relation of vaccine uptake and the outcome of interest:

SOURCE OF INFORMATION

Data on ILI cases were collected at the GP office level. GPs were interview the patients using a standardized questionnaire and added any relevant information present on the patients' records, such as vaccination status or chronic diseases (Annex). Data from cases were anonymized and sent by mail to the Department of Epidemiology of the National Institute of Health where it was centrally collected.

TARGET GROUP FOR VACCINATION

An individual was considered in the target group for vaccination if he belongs to at least one of the following groups:

- suffer from at least one of the chronic condition listed in the item above;
- age (≥60 years);
- pregnancy in the second and third trimester;
- occupation (health professional and care taker);
- caregiver or cohabitant of children with less than 6 months with chronic conditions

Table 1 – Confounding factor and effect modifiers collected

Variable	Definition
Chronic conditions	• Diabetes: if treated for insulin or non-insulin-dependent diabetes; • Cardiovascular disease (congenital heart disease, hypertensive heart disease, ischemic heart disease, chronic heart failure); • Chronic renal disease (chronic renal failure and nephrotic syndrome); • Chronic hepatic disease (cirrhosis, biliar atresia and chronic hepatitis); • Obesity IMC>=30; • Chronic respiratory disease (asthma, chronic bronchitis, emphysema, brochopulmonary dysplasia, cystic fibrosis, pneumoconiosis and pulmonary fibrosis) ; • Immunodeficiency congenital or acquired (conditions that suppress the immune function due to underlying disease and/or therapy, e.g. chemotherapy, HIV infection); • Neuromuscular disease.
Severity	The severity of the underlying conditions was measured by the number of hospital admissions due to underlying conditions in the 12 months prior to inclusion in the study.
Smoking	Smoking history was collected and coded as follows: never-smoker, former smoker (stopped smoking at least one year before inclusion in the study), current smoker.
Previous vaccinations	Vaccination against seasonal influenza in the last season (2013-2014).
Functional status	Low functional status on adults was defined as needing help to bath.
GP consultations	In order to document and control for access to care in the various control groups, the number of all GP visits in the 12 months before inclusion in the study was recorded.
Antiviral administration	Use of antivirals was documented: type and date of administration.

COLLECTED INFORMATION

Collected information was included (see also Annex 1: List of variables, definition and coding):

- study identification: country and GP;
- case/control demographics;
- ILI signs and symptoms;
- date of ILI onset;
- date of swabbing;
- laboratory results;
- patient is a health professional or care provider;
- selected underlying chronic conditions (including diabetes, heart disease, chronic obstructing pulmonary disorder, renal diseases and immunodeficiencies);
- obesity;
- number of hospitalisations for chronic diseases in the previous 12 months;
- total number of GP visits in the previous 12 months;
- smoking history;
- current season influenza vaccination including date and brand;
- influenza vaccination in the previous season;
- pregnancy status;
- functional status;
- antiviral administration

DATA VALIDATION

All paper questionnaires received were checked for missing values and inconsistencies, clarification and information recovered were done by e-mail or direct phone call to the GP.

The agreement between patient vaccine records and reported vaccination status by study participant /vaccine registries was validated, if possible.

Double data entry was performed. Inconsistencies were checked and corrected by comparison with paper record or by direct phone call to the GP. Consistency validation was systematically performed (e.g. the date of the interview must be later than the date of the beginning of the symptoms; case must meet the EU definition criteria).

DATA MANAGEMENT

Information from laboratory results were sent in structured database with patient identification code to the Department of Epidemiology by the National Influenza Reference Laboratory weekly.

Data entry was performed on a Microsoft Excel Database by typing in the answers from the questionnaires and laboratory results. Optical reading of the questionnaires also was performed.

SATISTICAL ANALYSIS

Descriptive and univariable analysis

Study participants were described by baseline characteristics. Baseline characteristics of cases and controls were compared using the chi-square test, Fisher's exact test, t-test or the Mann-Whitney test (depending on the nature of the variable and the sample size).

The association between vaccination status and baseline characteristics were measured for both case and control groups.

Measure of effect

Vaccine effectiveness was computed as VE = 1 – OR. An exact 95% confidence interval was computed around the point estimate. The vaccine effectiveness was calculated by virus strain and by lineage.

Stratified analysis

Analysis was stratified according to target group for vaccination

Multivariable analysis

Multivariable logistic regression analysis was conducted to control for negative and positive confounding. Odds ratio and standard errors were obtained. Interactions were tested using the likelihood ratio test or Wald's test and was included in the model if significant at the 5% level. Factors other than statistical significance were used as criteria for inclusion of a confounding factor or an interaction term.

ETHICAL ISSUES AND DATA PROTECTION

Given that no major chances were introduced in this protocol, compared to the 2011-12 protocol, the study protocol is already approved by the Comissão de Ética (Ethics Committee) do National Health Institute Doutor Ricardo Jorge and by the Comissão Nacional de Protecção dos Dados (National Committee of Data Protection).

The study protocol was approved (20th August 2012) by the Comissão de Ética da Administração Regional de Saúde de Lisboa e Vale do Tejo (Ethics Committee of the Regional Health Administration of Lisbon).

Results

INFLUENZA 2014/2015 SEASON

INFLUENZA EPIDEMIC DURATION

Clinical and laboratory data collected through the National Influenza Surveillance Programme (GP Sentinel Network - Rede Médicos-Sentinela, Network of Emergency Units and National Laboratory Network for Influenza Diagnosis) indicates that influenza epidemic occurred between week 1/2015 and week 8/2015 and had a medium-high intensity.

ILI INCIDENCE

Compared to the last season (2013/14), ILI incidence was higher in the present season with the highest ILI incidence observed in week 1/2015 (175.3 ILI cases by 100 000 inhabitants) (Figure 1).

VIRUS CIRCULATION

Influenza viruses circulating during the 2014-15 season, were detected since the week 45/2014, with a proportion of positive cases above 50% between weeks 52/2014 and 6/2015 (from late December through early February).

From week 50/2014 to week 9/2015, influenza B, A(H3) and A(H1)pdm09 viruses co-circulated. Influenza B was detected in higher proportion (37%), especially during the beginning of the season. Influenza A(H3) was detected in 16% of influenza cases and was more frequent in the second part of the epidemic period. A(H1)pdm09 was only detected sporadically and accounted 2% of influenza cases.

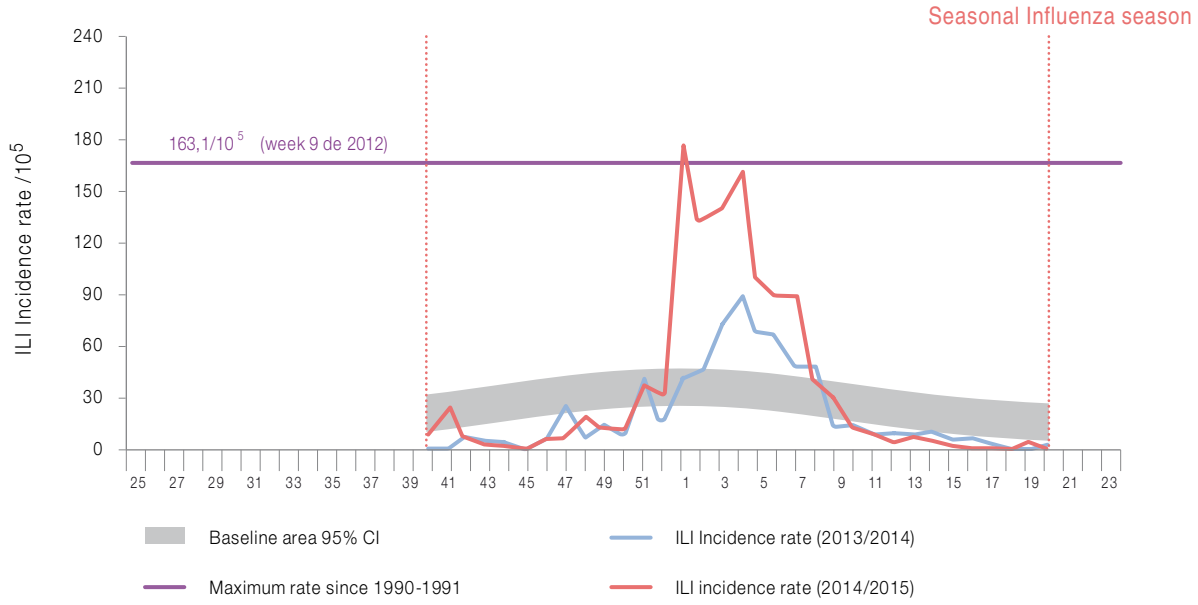

Figure 1 – Influenza like illness (ILI) incidence (/10^5 inhabitants) in Portugal, seasons 2013/14 and 2014/15.

EuroEVA 2014/2015 SEASON

STUDY PERIOD

ILI cases were selected by GPs starting on week 48/2014. As previously established in the scientific protocol, a period of 2 weeks with no positive cases for influenza, after the epidemic period, would determine the end of the study. Data collection ended at week 16, since from week 14 none of the enrolled ILI cases was positive for influenza.

PARTICIPATING GP'S

From the 50 GP's that accepted to participate in the study, 31 effectively participated in the study by selecting patients (which corresponds to a 62% participation rate). Each GP recruited in average 8.6 patients, in a total of 268 ILI cases.

All participating GPs work in a Health Center of the National Health Service (Ministry of Health) and have a stable list of patients. GPs that accepted to participate and reported ILI cases for the project were distributed by 14 of the 18 Districts of mainland Portugal and 1 Autonomous Region (Madeira) (Figure 2).

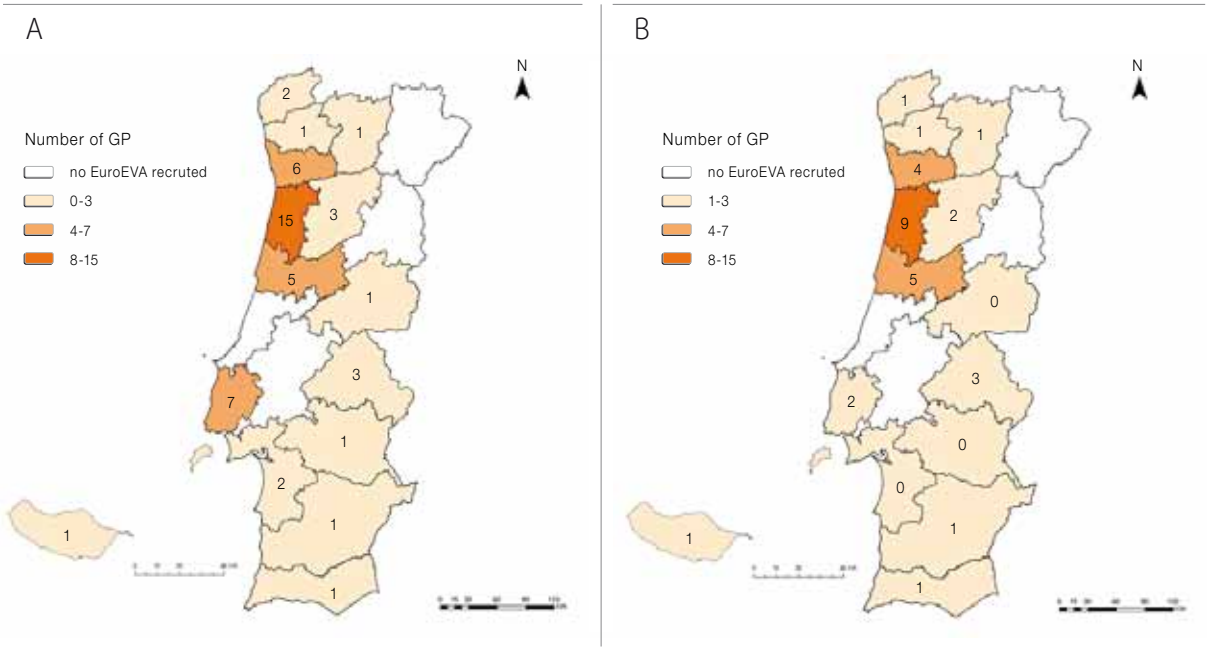

Figure 2 – Distribution of participating a) and effectively reporting b) GPs.

LABORATORY DIAGNOSIS AND CHARACTERIZATION OF INFLUENZA VIRUS

Nasopharyngeal swabs were collected for virological analysis at the National Influenza Reference Laboratory (see results by week of symptoms onset in Figure 3).

Laboratory analysis shows that 58% of ILI cases were associated with influenza virus infection. In 268 ILI cases, were found 105 influenza B/Yamagata viruses (39%), 48 influenza A(H3) (18%) and 2 influenza A(H1)pdm09 viruses (1%) (Figure 4). These percentages are similar to those found by the virological surveillance in the scope of the National Influenza Surveillance Program. However at European level A(H3) were detected in the beginning of the season and at higher percentage comparing to influenza B virus.

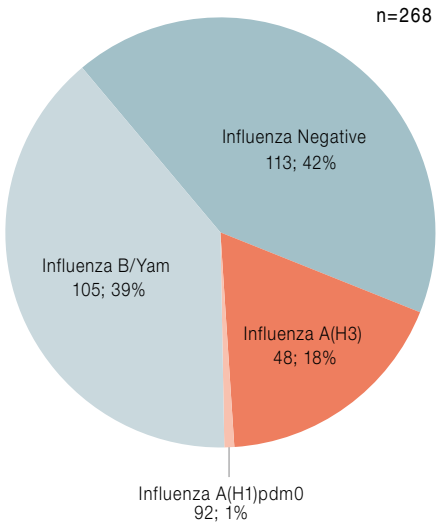

Figure 4 – Virological characterization of ILI cases, influenza virus type and subtype, during 2014/2015 season.

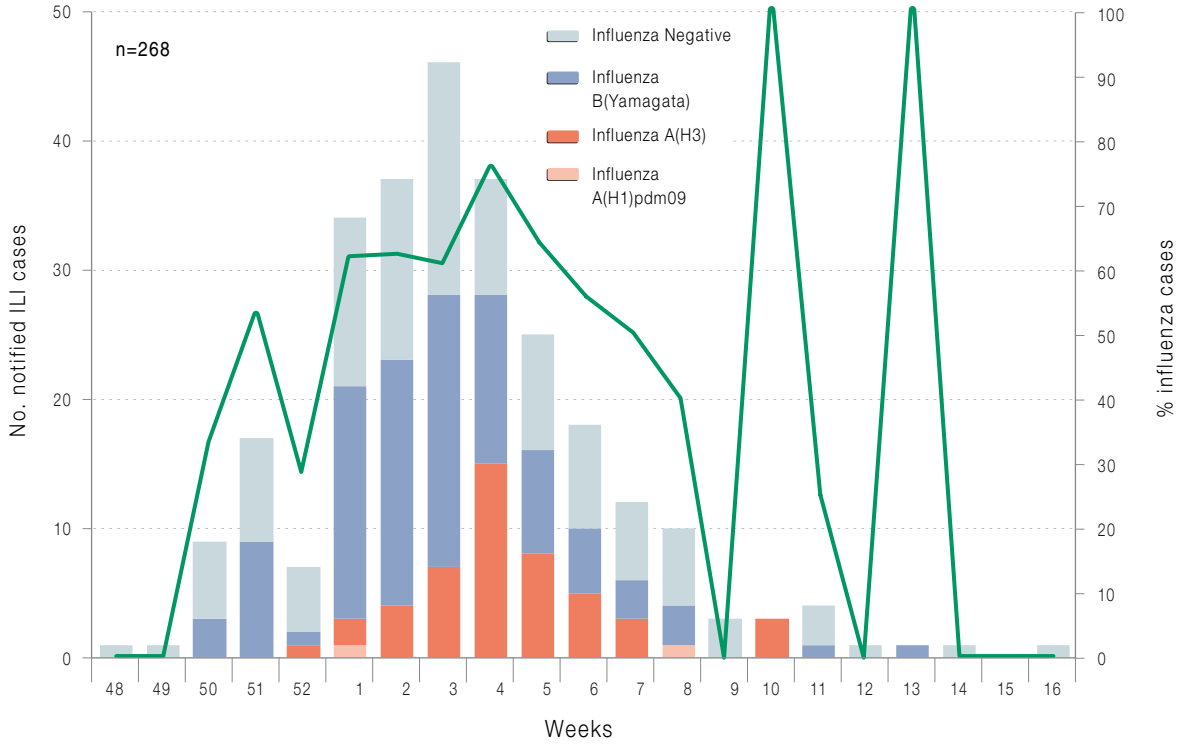

Figure 3 – Weekly distribution of the 268 analyzed ILI cases.

In the current season, the differential diagnosis for respiratory viruses was performed on EuroEVA ILI cases, negative for influenza. The multiplex PCR included detection of Respiratory Syncytial Virus (type A and B), Rhinovirus, Parainfluenza virus, Human Coronavirus, Adenovirus and Human Metapneumovirus. The results are shown in Figure 5.

Rhinovirus and RSV were the most frequent respiratory viruses after influenza, found in 22 (8%) and 8 (3%) ILI cases, respectively. Parainfluenza virus (4; 1%), Human Metapneumovirus (6; 2%), Human Coronavirus (3; 1%) were detected at lower frequencies. Only 1 mixed infection between Human Metapneumovirus and Human Coronavirus was detected.

All influenza positive samples were inoculated into cell-tissue culture (MDCK-SIAT1 cells) and 63 strains of influenza virus were isolated (Figure 6), 56 influenza B and 7 influenza A viruses (one H3 and one H1pdm09). Fourteen influenza A(H3) viruses presented a limited hemagglutination activity (HA titres under 8), although were detected in cell culture supernatant by neuraminidase activity assay. For that reason, the antigenic characterization of influenza A(H3) viruses was not performed by hemaglutination inhibition assay. The majority of type B viruses were more similar to 2015/2016 vaccine strain B/Phuket/3073/2013 (n=44) than to B/Massachusetts/02/2012 (2014/2015 vaccine strain). However, 12 influenza B viruses were poorly recognized by antisera raised against B/Massachusetts/02/2012 and B/Phuket/3073/2013 vaccine strains. The isolated influenza A(H3) virus was closer related with A/Switzerland/9715293/2013 than with the 2014/2015 vaccine strain A/Texas/50/2012. The H1 pandemic virus was antigenically similar to the vaccine strain

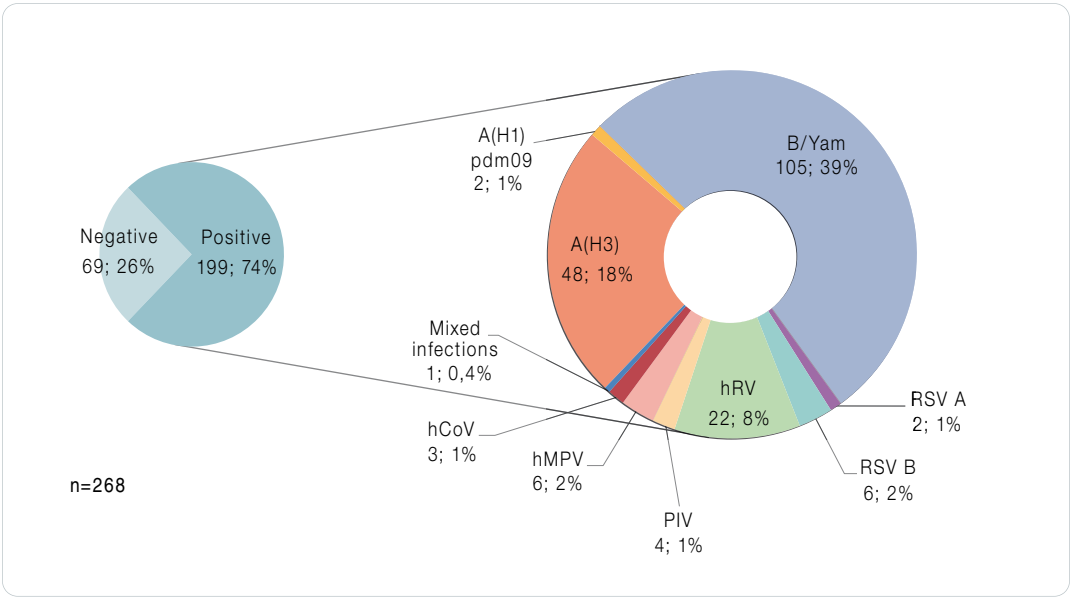

Figure 5 – Virological characterization of ILI cases. Combined laboratory results for influenza A and B, RSV A and B, rhinovirus, parainfluenza, Human coronavirus, adenovirus and Human metapneumovirus.

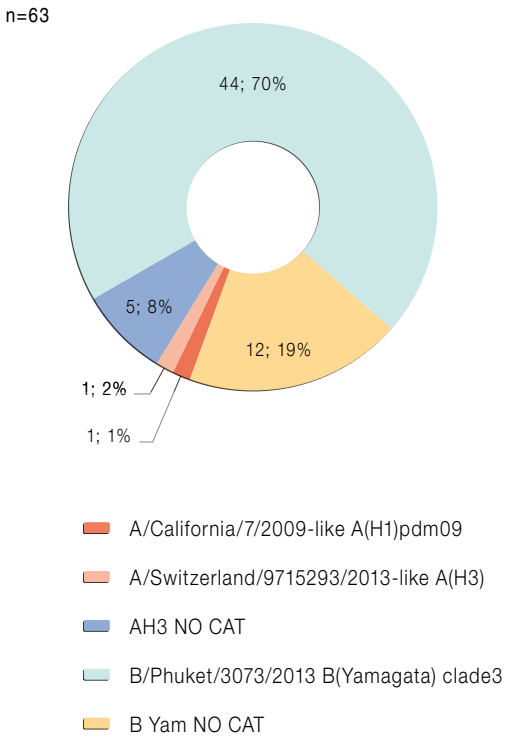

Figure 6 – Antigenic characterization of influenza viruses from EuroEVA 2014/2015 ILI cases.

A/California/7/2009 (Figure 7). Nine of these isolates [1 A(H1)pdm09 and 8 B/Yamagata] were also antigenically analyzed in WHOcc reference laboratory in London, results indicate that A(H1)pdm09 were similar to vaccine 2014/2015 virus and influenza B/Yamagata, although recognized by B/Massachussets/02/2012 antiserum, viruses were better recognized by antiserum raised against B/Phuket/3074/2013, selected for 2015/2016 season influenza vaccine.

Genetic analysis (Table 2, Figures 7-9) based on the HA1 subunit of the hemagglutinin gene was performed on 32 influenza viruses [19 A(H3), 9 B/Yamagata and 2 (H1)pdm09]. Influenza A(H3) viruses clustered into 2 genetic groups (Figure 7): 15 viruses in the group 3C.2a (A/Hong Kong/5738/2014) and 4 in the group 3C.3 (A/Samara/73/2013). The majority of the A(H3) viruses belongs to the genetic group 3C.2a that includes strains antigenically different from the vaccine stain. The eleven isolated B/Yamagata viruses belonged to the group 3 of this lineage represented by B/Phuket/3073/2013 (Figure 9). The 2 A(H1)pdm09 viruses genetically characterized (Figure 10) belonged to the group 6B (A/South Africa/3626/2013). Seven A(H3) sequenced viruses were detected in vaccinated ILI cases, 6 belonged to genetic group 3C.2a and 1 belonged to 3C.3 (Figure 7).

Table 2 – Genetic characterization of influenza viruses isolated from EuroEVA 2014/2015 ILI cases.

Subtype/Lineage	Phylogenetic group	N. of viruses
A(H1)pdm09	A/Hong Kong/5659/2012 (group 6A)	0
	A/South Africa/3626/2013 (group 6B)	2
	A/Dakar/04/2014 (group 6C)	0
A(H3)	A/Texas/50/2012 (subgroup 3C.1)	0
	A/Samara/73/2013 (subgroup 3C.3)	4
	A/Hong Kong/5738/2014 (subgroup 3C.2a)	15
B/Yamagata	B/Massachusetts/2/2012 (group 2)	0
	B/Phuket/3073/2013 (group 3)	11
Total of sequenced viruses		32

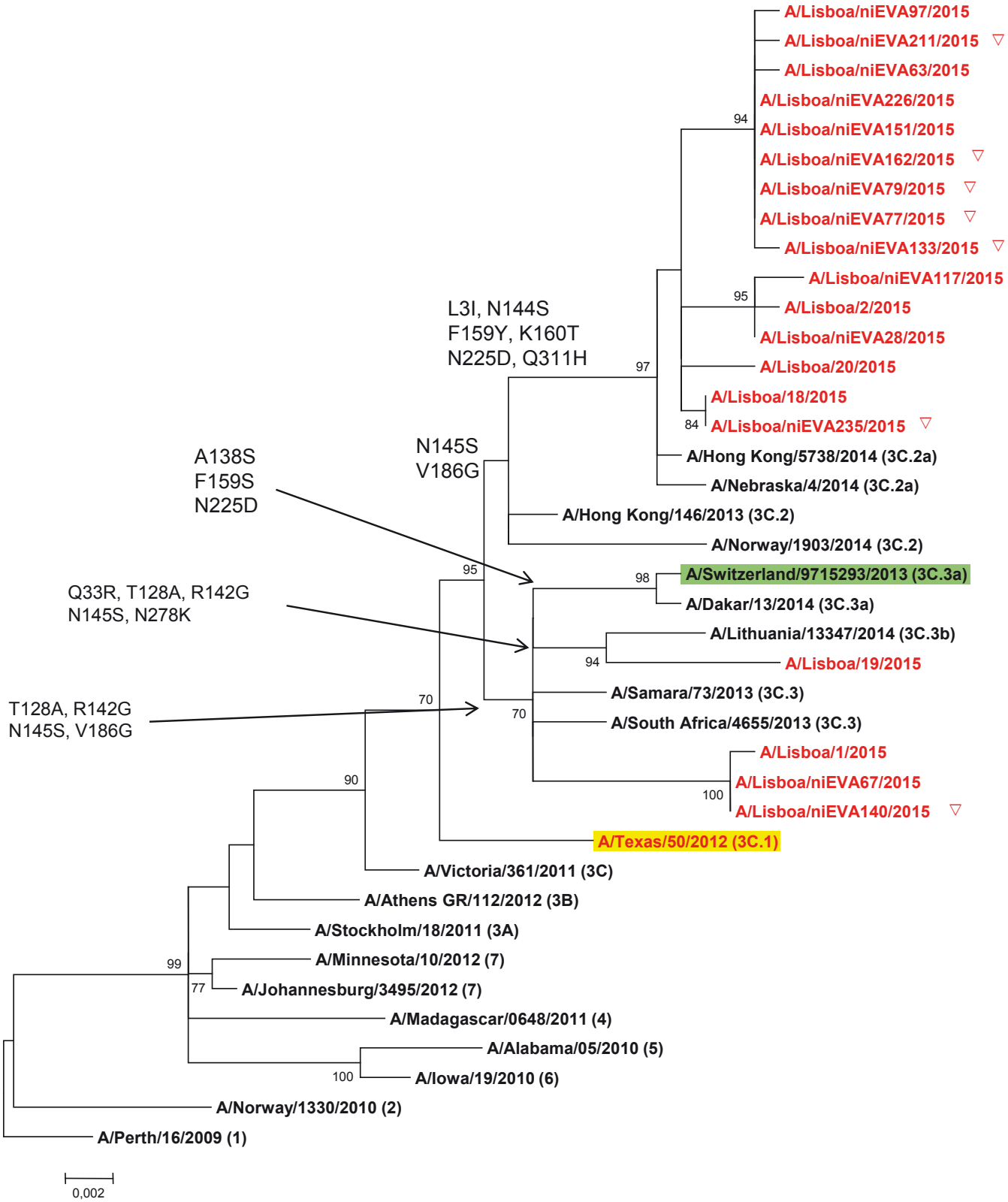

Figure 7 – Phylogenetic tree of influenza A(H3) viruses based on the HA1 subunit. Bootstrapp values above 70 are shown. Viruses detected and characterized in this study are shown in red. Reference strains are in black with respective genetic groups in brackets. The 2014/2015 and 2015/2016 northern hemisphere vaccine strains are highlighted in yellow and green, respectively. ▽ - viruses detected in vaccinated individuals.

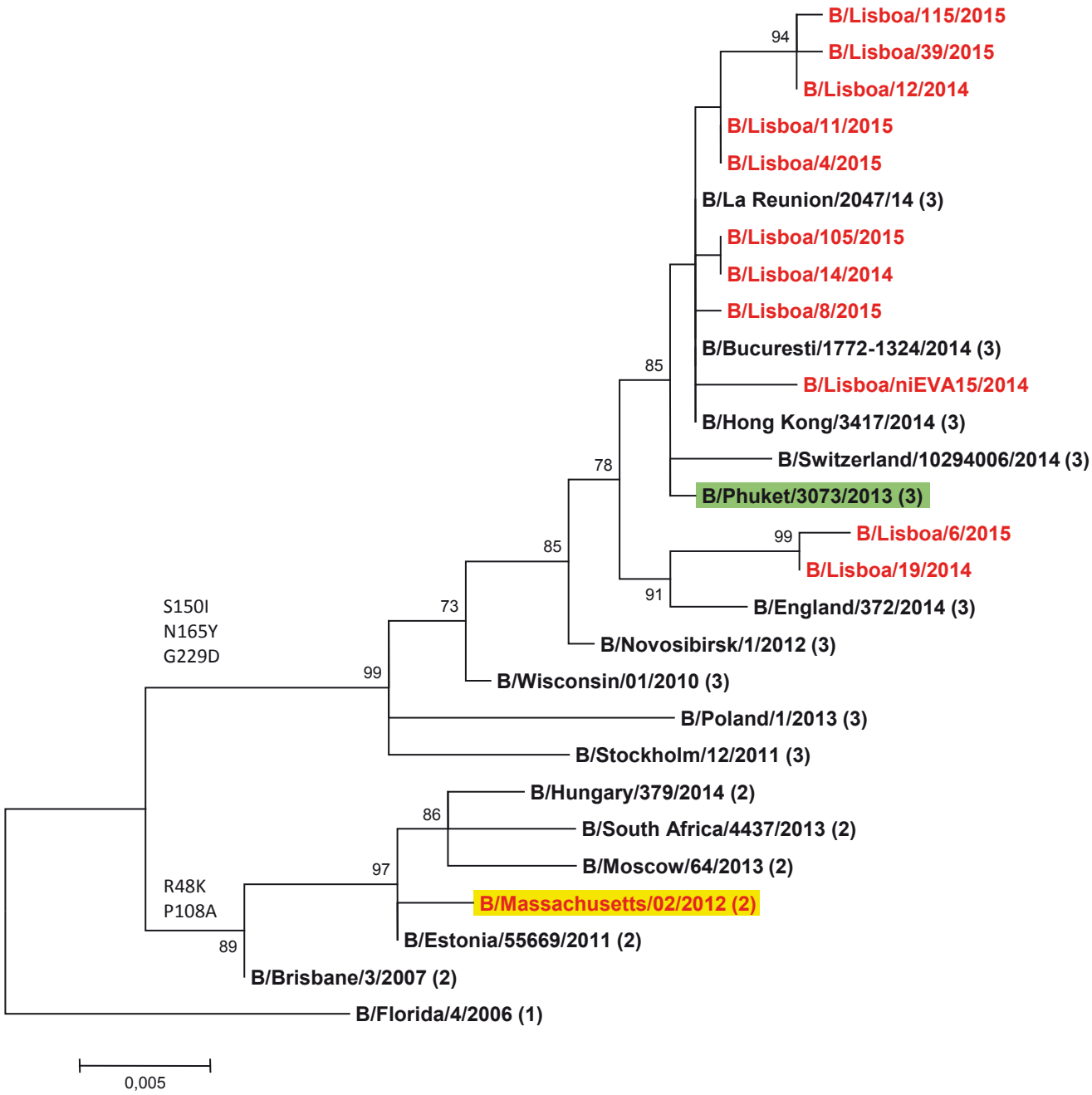

Figure 8 – Phylogenetic tree of influenza B/Yamagata viruses based on the HA1 subunit. Bootstrapp values above 70 are shown. Viruses detected and characterized in this study are shown in red. Reference strains are in black with respective genetic clades in brackets. The 2014/2015 and 2015/2016 northern hemisphere vaccine strains are highlighted in yellow and green, respectively.

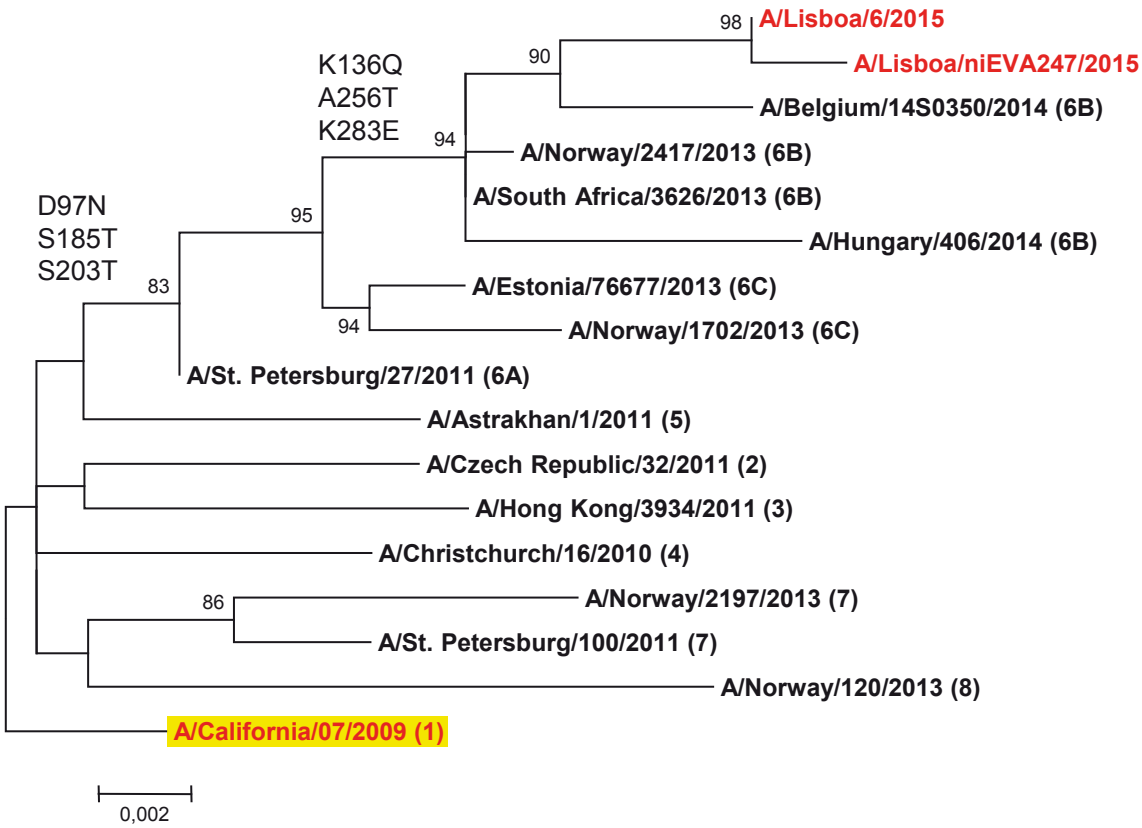

Figure 9 – Phylogenetic tree of influenza A(H1)pdm09 viruses based on the HA1 subunit. Bootstrapp values above 70 are shown. Viruses detected and characterised in this study are shown in red. Reference strains are in black with respective genetic groups in brackets. The 2014/2015 and 2015/2016 nothern hemisphere vaccine strain is highlighted in yellow.

DESCRIPTION OF PARTICIPANTS

A total of 268 ILI cases were selected by the participating GP's from week 48/2014 and 16/2015. The data set for analysis comprised 249 ILI cases. The flowchart of data inclusion/exclusion is presented in Figure 10.

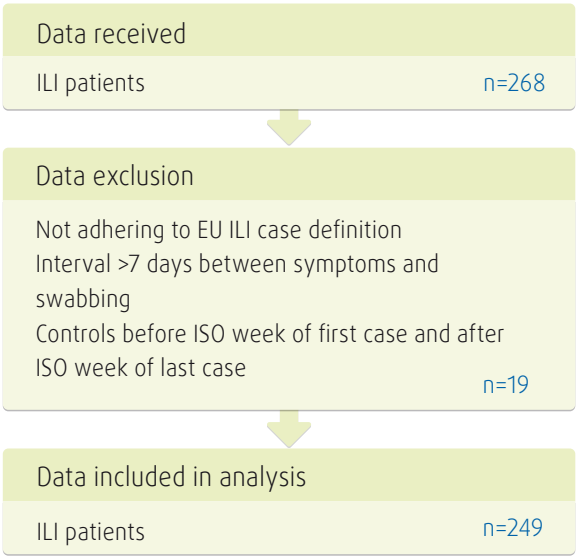

Figure 10 – Flowchart of data inclusion/exclusion.

DESCRIPTION OF CASES AND CONTROLS

After excluding 19 ILI cases (for not adhering to the inclusion criteria) the final sample consisted on 249 ILI patients. Of the 142 cases which tested positive for influenza, 67% were positive for influenza B/Yamagata, 32% for A(H3) and the remaining for subtype A(H1)pdm2009 (1 %).The control group, consisting of 97 ILI patients who tested negative for influenza, was statistically different ($p<0.05$) from the cases in the following variables (Table 2):

- **Age:** controls were older than cases (median age in controls was 51 yrs vs. 44 yrs in cases);
- **Any chronic disease:** the prevalence of at least one chronic condition relevant for influenza vaccination was higher in controls (44.1% vs 28.1%);
- **Seasonal vaccine in 2013-14:** controls were more often vaccinated against influenza in the last season than cases (28.4% vs. 12.3%);
- **Help bathing:** controls needed more help for bathing than cases (0% in cases vs 4.1% in controls).

Table 3 – Description of Cases and Control, from week 50 to week 10 during the 2014-2015 EuroEVA season.

	Influenza	Controls
Time between onset and swab collection (days)		
median	2 (147)	2 (102)
*p*1[a]	0.610	
less than 72h, %	84.4 (147)	76.5 (102)
p2[b]	0.118	
Age, mean	44.0 (147)	51.0 (102)
*p*1[a]	0.013	
0-4 years, %	1.4	3.9
5-14 years, %	11.6	2.9
15-60 years, %	66.7	56.9
≥60 years, %	20.4	36.3
*p*1[b]	0.003	
Sex, male %	42.2 (147)	44.1 (102)
*p*1[b]	0.647	
Smokers, %	9.5 (142)	13.73 (97)
*p*1[b]	0.302	
Seasonal vaccine 2013-14, %	12,3 (146)	28.4 (102)
*p*1[b]	0.002	
Chronic diseases (any), %	28.1 (146)	44.1 (102)
*p*1[b]	0.006	
Help for bathing, %	0.0 (146)	4.1 (100)
*p*1[c]	0.028	
Belongs GP patient list, %	66.7 (147)	70.6 (102)
*p*1[b]	0.513	
Vaccination target group, %	38.8 (147)	51.0 (102)
*p*1[b]	0.056	
GP consultations last 12 mo, median	2 (139)	3 (99)
*p*1[a]	0.081	
Any hospitalisations, %	2.0 (147)	3.0 (100)
*p*1[a]	0.631	
Years of education, median	6 (143)	6 (99)
*p*1[a]	0.751	
Co-habitants, median	2 (147)	2 (100)
*p*2[a]	0.011	

a: Mann-Whitney test; b: Chi-square test; c: Fisher' exact test

VACCINE COVERAGE

Considering all population, vaccine coverage (VC) in controls was 30.7%, statistically higher (p<0.001) than in cases (VC=11.0%). Similar results were obtained for the sub-group target for vaccination by the National Health Authorities (VC cases =26.8% and VC controls=54.9%, p=0.003) (Figure 11).

Restricting the analysis to type B virus (Yamagata lineage), VC was statistically (p=0.001) higher in controls than in cases (30.7% vs 8.1% in all ILI cases and 53.9% vs 20.0% in the target group) (Figure 11).

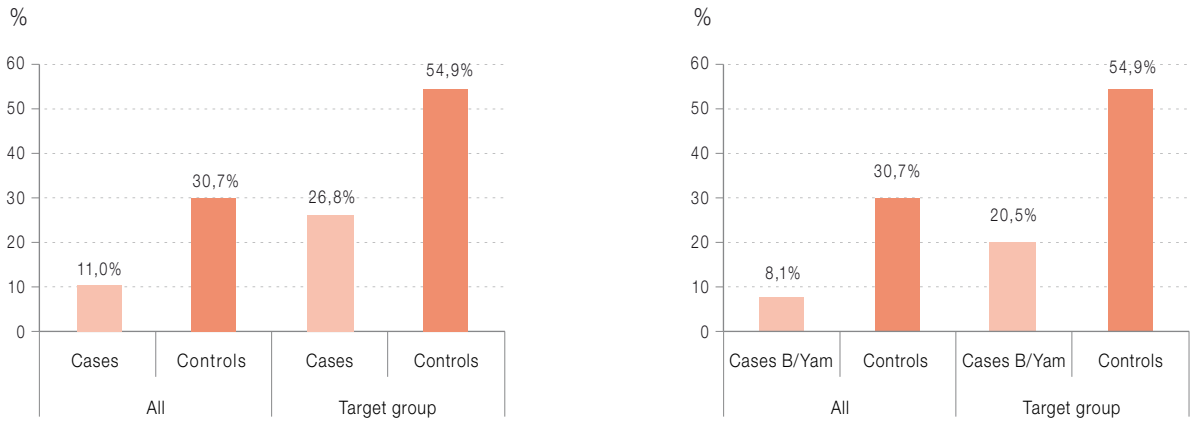

Figure 11 – Vaccine coverage in cases (all and B/Yam cases) and controls.

VACCINE EFFECTIVENESS

Given the vaccine coverage estimates, crude VE estimates was 72.2% (95% CI: 43.1%-86.7%) in all population and 69.9% (95% CI: (27.5%; 87.7%) in the target group for vaccination (Table 4).

Considering as a confounder all variables which adjusted for vaccine OR, compared to vaccine crude OR, changed more than 10% after M-H adjustment (Figure 12), age group and having any chronic disease were selected as confounders.

After adjustment for age, chronic disease and month of onset of illness, VE adjusted estimates in all population was 63.4% (95% CI: 16.2%; 84.0%). In the target group for vaccination, after adjustment, VE estimates was 65.0% (95% CI: 8.1%-86.7%). Restricting to B Yamagata cases, similar VE estimates were obtained for all population and target group (approximately 79%). All crude and adjusted VE estimates were statistically significant, but with low precision.

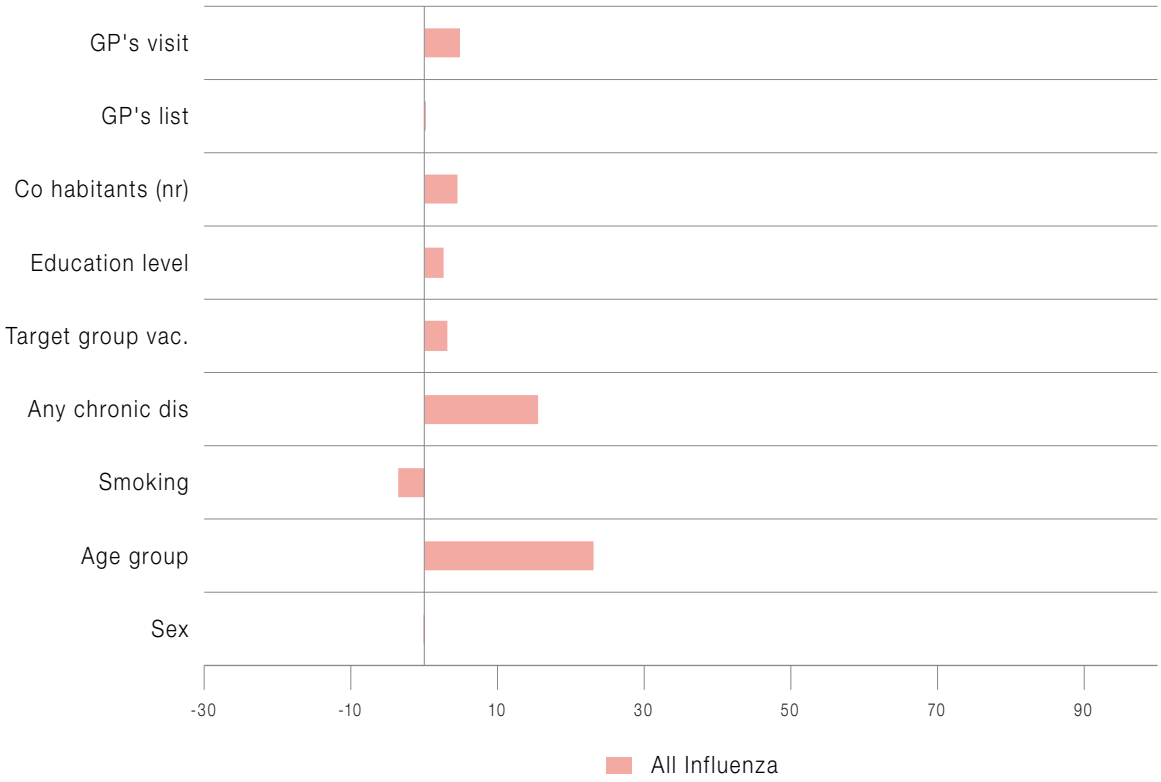

Figure 11 – Seasonal vaccine 2014-15 Odd Ratio change (%) after adjustment for potential confounding factors.

Table 4 – Vaccine effectiveness (crude and adjusted) in general population and in target group for vaccination.

	Crude		Adjusted	
	VE	CI95%	VE	CI95%
All population				
Influenza	72.2%	(43.1; 86.7)	63.4%	(16.2; 84.0)
B/ Yam	80.1%	(52; 92.5)	78.5%	(39.5; 92.4)
Target for vaccination				
Influenza	69.9%	(27.5; 87.7)	65.0%	(8.1; 86.7)
B/ Yam	78.6%	(40.1; 92.8)	79.6%	(34.7; 93.6)

Discussion and conclusions

Overall VE point estimates indicate that this season, the influenza vaccine conferred moderate protection against all influenza virus (around 64%) and adjusted VE estimates were similar for target group (63.7%) and general population (63.4%).

Restricted analysis to virus sub-type, revealed that VE was considerable high against B Yamagata lineage, around 79%, and although this was statistically significant, it was calculated with a higher imprecision than the desirable (95%CI varied between 40% and 90%). The low sample size did not allow VE estimates for the other sub-type virus in circulation, namely A(H3) and A(H1)pdm09.

When compared to other VE studies, we observed within EuroEVA higher overall estimates: France reported a VE of 32% (95% CI; 23%-40%) [7] among risk groups and US [8] published an even lower VE of 19% (95% CI; 7%–29%) for all individuals. One possible explanation for this low VE and discrepancy from our results may be related to the dominant influenza sub-type virus in circulation in these countries, A(H3). Even though no results were computed within EuroEVA data, for A(H3), preliminary results from the IMOVE pooled analysis and Canada [9] (VE=–8%; 95% CI: –50 to 23%) indicated also a low VE for this virus sub-type.

According to our results, the antigenic and genetic analysis of detected influenza viruses showed that influenza B and A(H3) viruses were different from vaccine 2014-2015 selected viruses. Influenza A(H1)pdm09, although detected sporadically, were similar to the vaccine strain. Antigenic analysis of influenza B viruses detected showed that majority of influenza viruses were B/Phuket/3073/2013-like, the strain selected for next season influenza vaccine and only one strain was B/Massachusetts/2/2012-like (2014/2015 vaccine strain). All influenza B genetically characterized viruses fall in the clade 3 (represented by B/Phuket/3073/2013), which presents characteristics amino acid substitutions in antigenic sites that distinguishes these viruses from the vaccine strain. Clade 3 influenza B viruses were circulating all over European countries especially in the end of influenza season, but predominantly in Portugal.

Even though influenza B genetic analysis revealed differences from the vaccine strain, all influenza B viruses detected belonged to the Yamagata lineage and were similar to the B/Massachusetts/2/2012 virus selected for 2014/2015 flu vaccine [10] and so, the high VE results could be explained by cross-reactivity between antibodies to different genetic clades or even between different lineages, as described by some studies [11].

Influenza A(H3) circulating viruses belonged to two different genetic clades , the majority fall in genetic subgroup 3C.2a that contain new drift variants compared to A/Texas/50/2012, selected strain for 2014/2015 north hemisphere influenza vaccine [10]. This situation was also observed all over European countries [12]. The antigenic analysis were performed to limited number of isolates, has most of the A(H3) viruses lost the ability to agglutinate red blood cells. This characteristic is

probably linked with amino acid substitutions in 159 and 160 positions (F159Y and K160T) that are present in majority of isolated viruses [13]. Viruses from clade 3C.2a bearing 10 substitutions (9 in antigenic sites) in hemaglutinin protein, comparing to vaccine strain, and antigenically distinct from vaccine strain, is consistent with a vaccine mismatch for A(H3) virus. This fact is in line with the low VE observed in the present season specially regarding to A(H3) circulating viruses [9] showing little or no protection for A(H3) predominant circulating strains.

In conclusion, the number of GP that participated in EuroEVA, their geographical distribution and the number of participants increased during this season (32 vs 23 in 2013/14). Also the average of ILI cases recruited by GP increased in this season (8.9 vs 2.9 in 2013/14). The improvement of these process indicators may be related to the reinforcement of resources allocated to the project. This effort was reflected on the sample size with 268 ILI patients enrolled (125 in 2013/14 season) enough to provide statistically significant for adjusted VE estimates against the dominant virus in circulation, B/Yamagata. The following key messages can be taken from EuroEVA in this season:

- The 2014-2015 season adjusted VE estimates against B/Yamagata was approximately 79% (statistically significant but with a lower precision than desirable);
- All influenza B belonged to genetic group 3, different from genetic group 2, represented by B/Massachusetts/02/2012 vaccine strain but antigenically these stains are related and this could explain the moderate/high protection provided by the vaccine
- No VE estimates against A(H3) and AH1pdm2009 were computed due to small sample size but influenza A(H3) viruses were genetically and antigenically different from the 2014/2015 vaccine strain, most of them belonging to the new virus cluster 3C.2a.

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