

_title:

Influenza vaccine effectiveness in Portugal

_subtitle:

Season 2015/2016 report

_edition:
INSA, IP

_authors: Department of Epidemiology, Department of Infections Diseases

Verónica Gómez, Raquel Guiomar, Ana Paula Rodrigues, Pedro Pechirra, Patrícia Conde, Paula Cristóvão, Inês Costa, Baltazar Nunes, Ausenda Machado

_place / date:
Lisbon
October 2016



Cataloging in publication:

PORTUGAL. Ministry of Health. National Health Institute Dr. Ricardo Jorge
Influenza vaccine effectiveness in Portugal : season 2015/2016 report / Department of Epidemiology, Department of Infections Diseases. -
Lisbon : National Health Institute Dr. Ricardo Jorge, 2016. - 48 p. : il.

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Title: Influenza vaccine effectiveness in Portugal: season 2015/2016 report

Authors: Department of Epidemiology (Verónica Gómez, Ana Paula Ambrósio Rodrigues, Baltazar Nunes, Ausenda Machado)
Department of Infections Diseases. National Influenza Reference Laboratory (Raquel Guiomar, Pedro Pechirra,
Patrícia Conde, Paula Cristóvão, Inês Costa)

Editor: National Health Institute Dr. Ricardo Jorge

Collection: Scientific and technical reports

Coordination editorial technique: Elvira Silvestre

Composition and pagination: Francisco Tellechea

ISBN (ebook): 978-989-8794-24-6

Lisbon, October 2016

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Instituto **Nacional de Saúde**
Doutor Ricardo Jorge





National Health Institute
Dr. Ricardo Jorge, IP

Av. Padre Cruz 1649-016 Lisbon
t: 217 519 200 @: info@insa.min-saude.pt

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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” and describes the results obtained in Portugal under the Protocol Agreement celebrated between EpiConcept SARL, Paris and National Health Institute Dr. Ricardo Jorge, Lisbon. Data and activities related to the individuals 65 years and more were funded by European Union’s Horizon 2020 research and innovation programme under grant agreement no 634446.

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SUMMARY

The results to be presented are related to the 8th EuroEVA season (the Portuguese component of the multicentre I-MOVE study) and aimed the estimation of 2015/2016 end of season influenza vaccine effectiveness in i) all age groups and in <65 and 65 plus years ; ii) by risk group; iii) by influenza subtype and clade.

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 adding a new variable related to statin uptake. Also, a pilot study to incorporate genetic characterization of influenza virus from patients enrolled in vaccine effectiveness (VE) study was implemented. Within this pilot study, influenza A(H1)pdm09 positive cases were randomly selected to be genetically characterized. Selection was conducted in three phases of the epidemic: early, peak and late season.

Results

In Portugal, a low intensity influenza epidemic occurred between week 53/2015 and week 8/2016. Influenza A(H1)pdm09 virus predominated during all season. Influenza B/Victoria lineage was detected in co-circulation in late season.

From the 84 GP's that accepted to participate in the study, 51 GP's effectively participated in the

study selecting patients (60.7% participation rate). A total of 336 ILI patients were enrolled and after excluding 26 ILI patients the final sample for analyses consisted on 310 ILI patients (147 cases and 102 controls). From the cases, 89.5% were positive for influenza A(H1)pdm09, 8.9% were positive for influenza B/Victoria and the remaining (1.6%) were positive for influenza A(H3). For genetic characterization of influenza virus, A(H1)pdm09 virus subtype was selected. The HA1 subunit of the hemagglutinin gene were successfully sequenced for 93 out of 126 cases with detected viruses. Phylogenetic analysis and clade assignment were performed. The majority of A(H1)pdm09 viruses belonged to the subgroup 6B.1 (87.1%) and the remaining viruses belonged to 6B clade. Comparing cases and controls, was confirmed that they were statistically different in relation to time between onset and swab collection, sex, seasonal vaccine uptake in 2014/2015, presence of at least one chronic disease and number of GP consultations in last 12 months.

For 2015/2016 trivalent influenza vaccine, confounder-adjusted VE against A(H1)pdm09 was 54.0% (95% CI: -1.5; 79.2%) in **all population** and 63.9% (95% CI: (7.7%; 85.9%) in the **target group** for vaccination.

Considering the population with **less than 65 years**, VE against AH1pdm09 was 56.2% (95% CI: -17.5%; 83.7%) and 74.9% (95% CI: -37.1; 95.4%) in the **65 and more years of age**.

Conclusions

VE estimates during 2015/2016 season indicated that the seasonal flu vaccine conferred moderate protection against A(H1)pdm09 (varied between 54% to 64% considering all population and the target group for vaccination).

The vaccine had a better performance in the elderly, where VE point estimates reached 75% (not significant nevertheless).

The implementation of the genetic characterization pilot study was well succeeded, with a success rate of influenza virus characterization of 80.2%. For future seasons, the high sampling fraction in the first phase should continue in order to assist the definition of the virus subtype/clade target during the season.

RESUMO

Os resultados apresentados correspondem à implementação da 8ª época do EuroEVA (a componente portuguesa do estudo multicêntrico I-MOVE) e pretende obter estimativas da efetividade da vacina (EV) sazonal 2015/2016 i) em todos os grupos etários e no grupo com idade inferior a 65 e com 65 e mais anos; ii) por grupo-alvo da vacinação e iii) por subtipo de vírus da gripe e por clade.

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 implementado na íntegra, acrescentando um nova variável relacionada com a toma de estatinas. De igual modo, foi implementando um estudo-piloto relacionado com a incorporação da caracterização genética dos participantes no estudo da efetividade. Dentro deste estudo-piloto, os casos positivos de A(H1)pdm09 foram aleatoriamente selecionados para caracterização genética, seleção esta que foi conduzida nas três fases do período epidémico: início, pico e fim.

Resultados

Em Portugal o período epidémico ocorreu entre as semanas 53/2015 e 8/2016 tendo-se verificado uma atividade gripal de intensidade baixa. Predominou a circulação de vírus do subtipo Influenza A(H1)pdm09 e o vírus Influenza da linhagem B/Victoria co-circulou no final da época.

De entre os 84 médicos de família (MF) que aceitaram participar no estudo, 51 reportaram doentes com síndrome gripal (SG), correspondendo a uma taxa de participação de 60,7%. No total foram selecionados 336 doentes com SG. Após exclusão de 26 doentes, a amostra final consistiu em 310 doentes com SG (147 casos e 102 controlos). De entre os casos, 89,5% eram do subtipo A(H1)pdm2009, 8,9% eram positivos para o vírus do subtipo B/Victoria e os restantes (1,6%) para o tipo A(H3). Para efeito de caracterização genética, o subtipo A(H1)pdm09 foi selecionado. A subunidade HA1 do gene da hemaglutinina foi sequenciada com sucesso para 93 dos 126 casos. A maioria dos casos de A(H1)pdm09 pertencia ao subgrupo 6B.1 (87,1%) e os restantes ao subgrupo 6B.

Comparando casos e controlos verifica-se que os grupos eram estatisticamente diferentes no que diz respeito, ao tempo entre início de sintomas e colheita, ao sexo, a toma da vacina na época anterior, à presença de doença crónica relevante para vacinação da gripe e ao número de consultas com o MF nos últimos 12 meses.

Para a vacina trivalente antigripal 2015/2016 a EV ajustada para confundimento contra o subtipo A(H1)pdm09 foi 54,0% (IC95%: -1,5; 79,2%) na população em geral e 63,9% (IC95%: (7,7%; 85,9%) no grupo alvo da vacinação contra a gripe.

Considerando a população com menos de 65 anos, a EV contra o subtipo A(H1)pdm09 foi de 56,2%

(IC 95%: -17,5%; 83,7%), sendo que foi de 74,9% (IC 95%: -37,1; 95,4%) no grupo com 65 e mais anos de idade.

Conclusões

As estimativas da EV da época 2015/2016 indicam que a vacina conferiu proteção moderada contra o subtipo A(H1)pdm09 (variou entre 54% e 64%, para a população em geral e para o grupo alvo da vacina, respetivamente).

A vacina parece demonstrar uma melhor performance na população mais idosa, onde as estimativas da EV alcançam os 75% (apesar de não estatisticamente significativas).

A implementação do estudo piloto foi bem conseguida, com uma taxa de sucesso de caracterização genética de 80,2%. Em épocas futuras, a amostragem na primeira fase deve manter elevada, de modo a assistir à definição do subtipo/clade do vírus a ser caracterizado.

1

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 Dr. Ricardo Jorge (INSA) in Portugal, conducted during the 2005/2006 and 2006/2007 seasons, two pilot studies with a cohort design¹. They were designed to provide data from sources independent of health services in order to allow the feasibility of the real study during a pandemic, if and when hospitals, health centres, physicians and other health services could collapse. Main conclusions drawn from these two pilot-studies stressed that estimation of effectiveness of 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 study design to estimate influenza vaccine effectiveness in seasonal and pandemic influenza seasons. INSA 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 case-control design able to measure in-season and end of the season influenza vaccine effectiveness, during the autumn and winter 2008-2009, among people aged 65 years and above, using several control groups².

Since the 2009/2010 season until present the study population started to include all individuals but with special interest in the target group for vaccination and 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”.

In this season, Portugal received funding from European Union’s Horizon 2020 research and innovation programme, for activities related to the 65 and plus age group, hence, in this report the results will be presented in all age groups and stratified in accordance.

Regarding the wide genetic and antigenetic variability of influenza viruses, overall or subtype estimated VE may not be sufficient to assess vaccine protection against circulating strains. For this reason, to attempt the subtype-specific VE estimates variations, for the first time, Portugal alongside with other 6 countries pilot-tested the integration of virological data in VE using a method to randomly select influenza positive specimens for genetic characterization.

2

Objectives

This report presents the results of the estimation of 2015/2016 end of season influenza vaccine effectiveness, that are related to the 8th EuroEVA season (the Portuguese component of the multicentre I-MOVE study).

This season the case-control test negative design was implemented once again and aimed at estimating influenza vaccine effectiveness by:

- age group (under 65 years old and 65 and over);
- target group;
- influenza type/subtype;
- by genetic clade.

3

Materials and methods

3.1 Study design, population and period

Study design

Case-control test negative design (Test Negative Design) where cases laboratory confirmed influenza-positive patients (ILI+) were compared to controls 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 catchments area of the participating General Practitioners (GP).

Study period

In order to estimate seasonal VE, ILI patients from all ages were selected by GPs starting on final of November 2015 (week 49/2015) and finished in early May 2016 (week 17/2015).

The peak of the epidemic was determined through the Portuguese routine surveillance system, "Médicos-Sentinela Network"³. The national influenza vaccination campaign started in week 40/2015.

3.2 Outcome

The outcome of interest was laboratory-confirmed influenza, by type, subtype, lineage and by clade.

3.3 Definitions on ILI patients, influenza cases and controls

ILI patients

A patient with influenza like illness (ILI) was defined as an individual who consults a participating GP, presenting ILI signs and symptoms (using EU ILI case definition⁴).

Influenza case

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

Controls

ILI patients that tested negative for any influenza virus [A(H1)pdm09, A(H3N2) and B] were included in control group.

3.4 Sampling

ILI patient identification

Cases were identified among patients that present with ILI to a participating GP. All participating GPs worked in a Health Centre of National Health Service (Ministry of Health) and had a stable list of patients. The participating GPs covered all Mainland Portugal and Autonomous Regions, as fit as possible. GPs recruited ILI cases from all ages (belonging or not to their patient list) in consultation setting.

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. This threshold in age was implemented given the recommendations from the General Directorate for Health⁵.

The systematic sampling procedure applied to patients <60 years, 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 randomly assign (e.g. for GP1 the “week” starts at Thursday, GP2 Tuesday, GP3 Monday, etc.).

Case and controls inclusion and exclusion criteria

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

Cases and controls 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.

Specimen selection for genetic characterisation

In order to define the minimum number of strains to be characterized, a sampling fraction was computed. Selection was performed in three phases established according to influenza activity:

1. **Early phase:** From first influenza case detected until the second week in which ILI weekly incidence is above the baseline threshold (05/12/2015 to 10/01/2016);

2. **Peak:** From third week above the ILI incidence baseline threshold until two weeks after the ILI peak (11/01/2016 to 31/01/2016);

3. **Late phase:** From third week after the ILI peak until the last positive case detected in the season (01/02/2016 to 01/05/2016).

Cases were allocated to each phase according symptoms to date of onset. At the end of each phase, viruses were selected using the Bernoulli sampling method, ensuring that each strain had the same probability of being selected.

3.5 Exposure (vaccination)

Definition of vaccination status

An individual was considered as vaccinated against influenza if the vaccination occurred more than 14 days before ILI symptoms onset. Seasonal vaccines available in Portugal, in 2015, 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 2015/2016 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.

3.6 Definition of risk groups

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

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 [Table 1](#);

- 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.

3.7 Confounding factors and effect modifiers

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

Table 1 – Collected confounding factor and effect modifiers.

Variable	Definition
Chronic conditions	<ul style="list-style-type: none"> ■ 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 $IMC \geq 30$; ■ Chronic respiratory disease (asthma, chronic bronchitis, emphysema, bronchopulmonary dysplasia, cystic fibrosis, pneumoconiosis and pulmonary fibrosis) ; ■ Congenital or acquired immunodeficiency (conditions that suppress the immune function due to underlying disease and/or therapy, e.g. chemotherapy, HIV infection); ■ Neuromuscular disease.
Severity	Severity was measured by the number of hospital admissions due to underlying chronic 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 (2014/2015).
Functional status	Low functional status on adults was defined as needing help to bath.
GP consultations	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.
Statin	Use of statin 30 days before de vaccine uptake.

3.8 Source of information

Epidemiological and laboratory data on ILI cases was collected at the GP office level. GPs interviewed patients using a standardized questionnaire and added any relevant information present on the patients' records such as vaccination status or chronic diseases (Annex 1). Data from cases and nasopharyngeal swabs were collected by the GP, anonymized and sent by mail to the Department of Epidemiology of the National Institute of Health where it was centrally gathered.

3.9 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 Dr. Ricardo Jorge.

Specimen collection, storage and transport

Nasopharyngeal swabs were collected into a suitable transport medium and sent to the laboratory. Sampling 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 4°C 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 seasonal influenza routine surveillance.

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 diagnosis of influenza infection was performed by real-time multiplex RT-PCR and was also conducted influenza viruses isolation in MDCK cell line (Mardin Darby Cell Kidney).

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 antigenically characterized by haemagglutination inhibition assay (HAI), carried out using antisera and reference virus strains provided by WHO Collaborating Center (London) ⁶. A selected sample of Portuguese isolates was sent to the WHO Collaborating Centre, in London, for further study.

The rapid detection and typing / 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. The RT-PCR for typing and subtyping of influenza viruses is accredited by Instituto Português de Acreditação (IPAC) ⁷. 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 cell line, allowing further antigenic char-

acterization of isolates and drug susceptibility assays ⁶.

For the influenza virus isolation, all swabs influenza positive by PCR, from period of recruitment, were inoculated in MDCK 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.

The National Reference Laboratory for Influenza follows internal control procedures and participates in external quality assessment programs organised by European Influenza Surveillance Network (EISN) and by the World Health Organization (WHO).

Negative swabs for influenza were also tested for other respiratory viruses by multiplex PCR: respiratory syncytial virus, human rhinovirus, parainfluenza virus, coronavirus, human metapneumovirus. The differential diagnosis of other respiratory agents will add value in identification of aetiological agent of the ILI syndrome.

Antigenic characterization

Isolates were characterized antigenically by haemagglutination inhibition assays (HAI), carried out using antisera and reference virus strains distributed by WHO Collaborating Center (London) ⁶. Selected isolates were sent to the WHO Collaborating Centre in London for further study.

Isolated viruses were compared to the circulating and vaccine strains selected for 2015/2016 northern hemisphere influenza vaccine.

Genetic characterization

The phylogenetic analysis of the coding region of the HA1 subunit of the hemagglutinin, was performed using the ClustalW Method for the multiple alignment and the maximum Likelihood Method for the construction of the phylogenetic trees (MEGA Software).

Description of antigenic/genetic mutations of viruses included

The antigenic characterization was based on virus interactions with post-infection ferret antisera raised against a vaccine influenza virus. HAI was performed using guinea pig red blood cells. A virus was considered similar to the vaccine strain if its HI titre with the antisera raised against the vaccine virus didn't differ by no more than 4-fold compared with the HI titre of antisera with the homologous viruses. A virus is considered different from a vaccine strain if the HI titre with the antisera raised against the vaccine virus differ by 8-fold or more, compared with the HI titre of antisera with the homologous viruses ⁸. The highest dilution of serum that prevents hemagglutination is called the HAI titer.

Viruses' genetic characterization and assigning to a specific genetic group were supported by phylogenetic and sequence analyses. Sequenced viruses were compared to viral reference sequences that represented each genetic group for influenza type/subtype and influenza B lineage⁸. A virus was ascribed to a specific genetic group if the phylogenetic analysis of the HA1 subunit of the hemagglutinin cluster within the clade represented by the vaccine/ reference virus and if it didn't contain many critical amino acid substitutions that

affect the antigenicity when compared to viruses that belonged to the group with which it associated.

3.10 Data collected

Collected information

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

EPI data

Data on ILI patients were collected at the GP office level. GPs 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 1](#)). 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. Collected epidemiological information included (see also [Annex 2](#): 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 (presented in [Table 1](#));
- 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;
- statin uptake status;
- pregnancy status;
- functional status;
- antiviral administration.

LAB data

The results of laboratory diagnosis for influenza, performed in each swab, were reported by the National Influenza Reference Laboratory to the GP that selected the case for study enrolment, in a standard INSA report sent by mail.

3.11 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.

Data validation

All received paper questionnaires were checked for missing values and inconsistencies. Data clarification and information recovery was made through e-mail or direct phone call to the GP.

Consistency validation was systematically performed. Inconsistencies in both databases were

checked and compared through a statistical programmed script.

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

3.12 Statistical 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 also calculated by virus type/subtype and genetic clade.

Stratified analysis

Analysis was stratified according to target group for vaccination and age group.

Multivariable analysis

Multivariable logistic regression analysis was conducted to control for negative and positive confounding. Odds ratio were obtained. Confounders

were included if crude OR changed more than 10% after Mantel-Haenszel adjustment.

3.13 Supervision

A supervising committee was established with participating members of the DGS (General Directorate of Health), INFARMED (National Authority of Medicines and Health Products), CEFAR/ANF (National Pharmacies Association), APMGF (Portuguese Association of General Practitioners), SPP (Pneumology Portuguese Society) and SPMS (Shared Services of the Portuguese Ministry of Health).

Two meetings were held, one in the beginning of the season and another one in the end of the season with the presentation of the study final results.

Ethical issues and data protection

Given that no major changes were introduced in this protocol, compared to the 2011/2012 protocol, the study protocol is already approved by the Ethics Committee of National Health Institute Dr. Ricardo Jorge.

The study protocol was re-approved for 2015/2016 season (30th June 2015) by the National Committee of Data Protection and approved (20th August 2012) by the Ethics Committee of the Regional Health Administration of Lisbon and Vale do Tejo.

4

Results

4.1 Influenza 2015/2016 seasons

Influenza epidemic duration

Clinical and laboratory data collected through the GP Sentinel Network “Rede Médicos-Sentinela” indicates that influenza epidemic occurred between week 1/2016 and week 9/2016 with low intensity during all season.

ILI Incidence

ILI incidence was lower than that observed during last season. The highest ILI incidence rate (59,4 by 105 inhabitants) was observed during week 3/2016 (Figure 1).

Virus circulation

Influenza viruses circulating during the 2015/2016 season, were detected since the week 45/2015, with a proportion of positive cases above 50% between weeks 2/2016 and 4/2016 (during January 2016).

Influenza A(H1)pdm09 viruses have largely predominated during the season (91%), however influenza B (8%) and A(H3) (1%) viruses were sporadically detected.

Since week 9/2016 was observed an increase in Influenza B detection (most from the Victoria lineage).

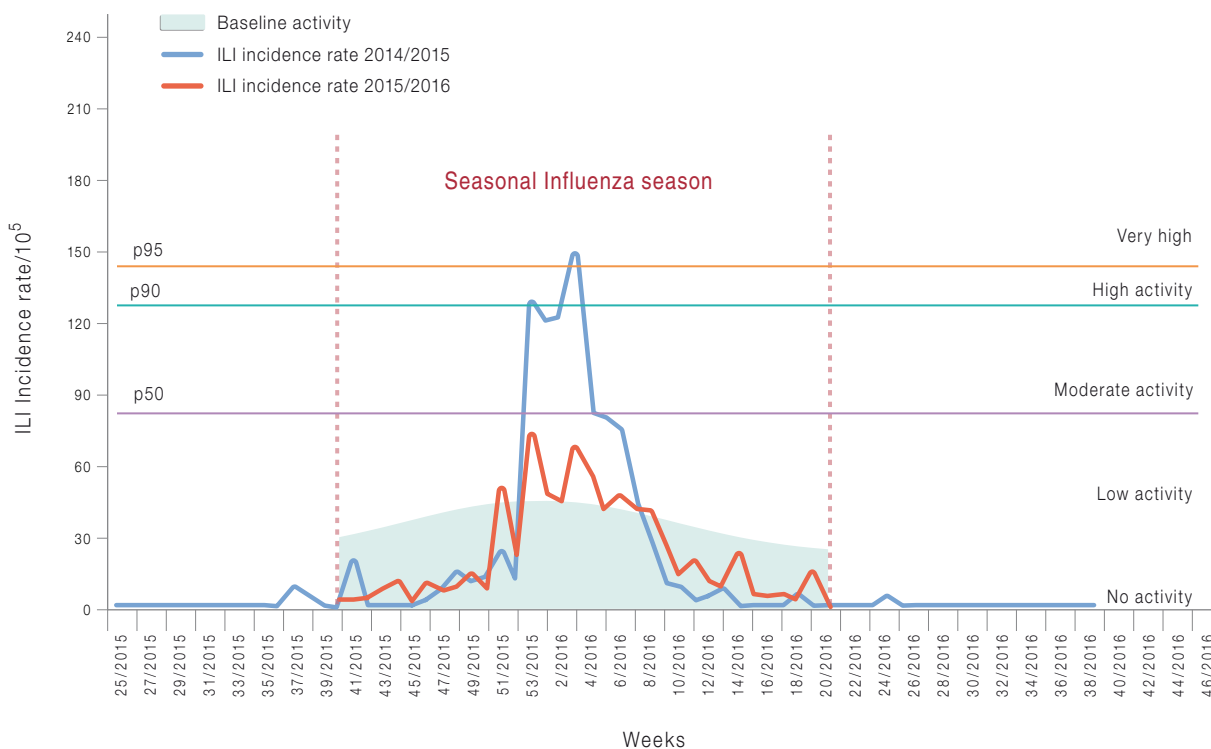


Figure 1. Influenza like illness (ILI) incidence (/10⁵ inhabitants) in Portugal, seasons 2014/2015 and 2015/2016.

4.2 EuroEVA 2015/2016 season

Study period

ILI cases were selected by GP starting on week 48/2015 and ended at week 17/2016.

Participating GP

From the 84 GP that accepted to participate in the study, 51 effectively participated in the study through patient's selection (which corresponds to

a 60.7% participation rate). Each GP recruited in average 6.6 patients (min: 0; max: 41), in a total of 336 ILI cases.

All participating GP work in a Health Centre of the National Health Service (Ministry of Health) and have a stable list of patients. GP that accepted to participate and reported ILI patients for the project were distributed by 15 of the 18 Districts of mainland Portugal and the two Autonomous Regions (Madeira and Azores) (Figure 2).

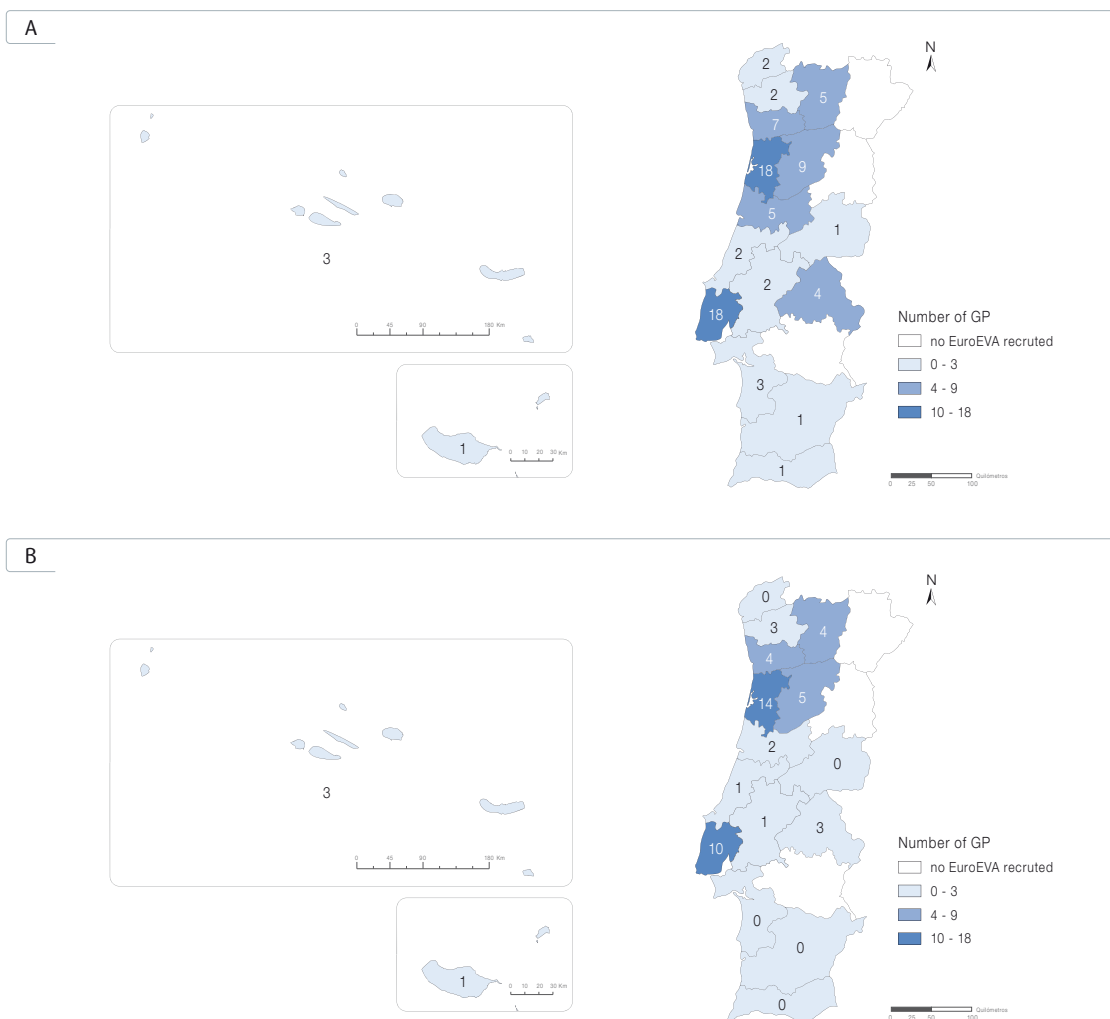


Figure 2 - Distribution of a) participating and b) effectively reporting GPs.

Laboratory diagnosis and characterization of influenza virus.

Nasopharyngeal swabs were collected and sent to the National Influenza Reference Laboratory for virological analysis. Influenza positive ILI cases were detected between week 49/2015 till week 17/2016. Influenza A(H1)pdm09 were detected mainly during the early and epidemic periods. Influenza B, were identified at low number in the last period of the season (see results by week of symptoms onset in Figure 3).

Laboratory analysis shows that 39.9% of ILI cases were associated with influenza virus infection. In 336 ILI cases, were found 117 (34.8%) influenza A(H1)pdm09 viruses, 12 (3.6%) influenza B/Victoria and 2 (0.6%) influenza A(H3) vi-

ruses (Figure 4). These percentages are similar to those found by the virological surveillance in the scope of the National Influenza Surveillance Program.

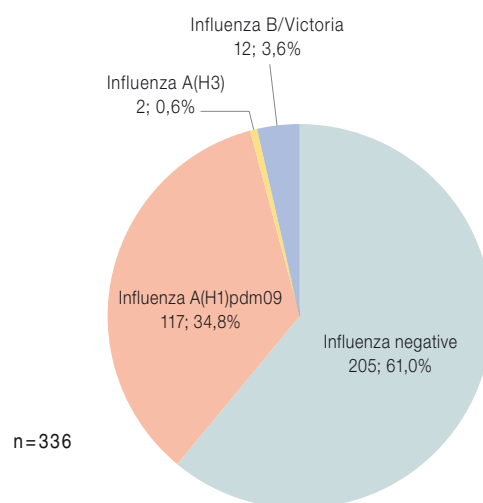


Figure 4 - Virological characterization of ILI cases, influenza virus type and subtype, during 2015/2016 season.

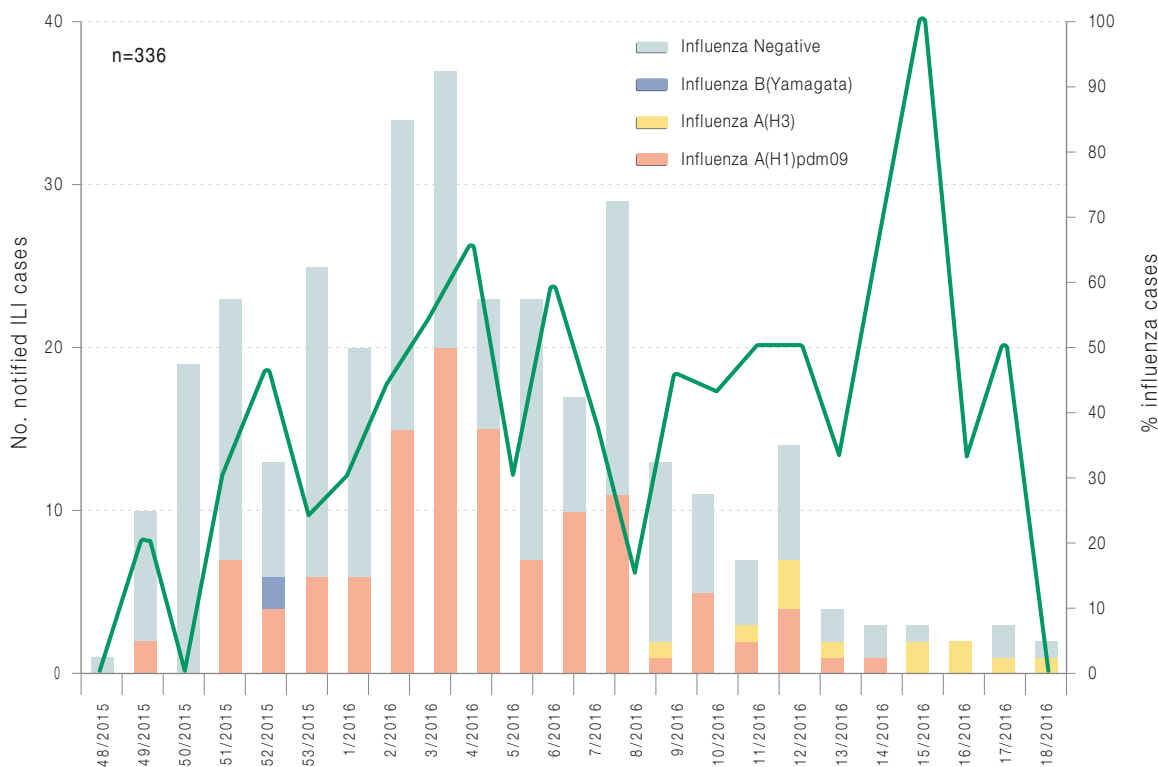


Figure 3 - Weekly distribution of the 268 analyzed ILI cases.

Human Coronavirus and Rhinovirus were the most frequent respiratory viruses identified in influenza negative swabs. Human Coronavirus and Rhinovirus were found in 39 (11%) and 38 (11%) ILI cases, respectively. Other viruses were also detected, at low frequencies: RSV (11; 3%), Human Metapneumovirus (9; 3%) and Parainfluenza virus (3; 1%). Were detected 2 mixed infections, one between Rhinovirus and Parainfluenza virus type 2, and other co-infection between Human Coronavirus and Human Metapneumovirus.

All influenza positive samples were inoculated into cell-tissue culture (MDCK cells) and 68 strains of influenza virus were isolated: 57 influenza A(H1)pdm09, 9 influenza B/Victoria viruses and 2 influenza A(H3) viruses (Figure 6).

All isolates A(H1)pdm09 were antigenically similar to the vaccine strain A/California/7/2009. Detected

influenza B viruses belonged to Victoria lineage, not represented in the 2015/2016 influenza vaccine composition. Influenza B/Victoria isolates were also antigenically different from the reference B/Victoria strain, B/Brisbane/60/2008.

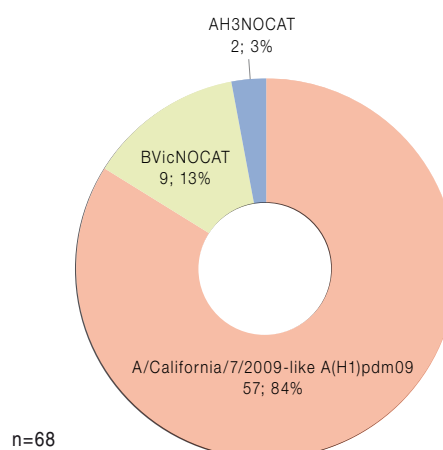


Figure 6 – Antigenic characterization of influenza viruses from EuroEVA 2015/2016 ILI cases.

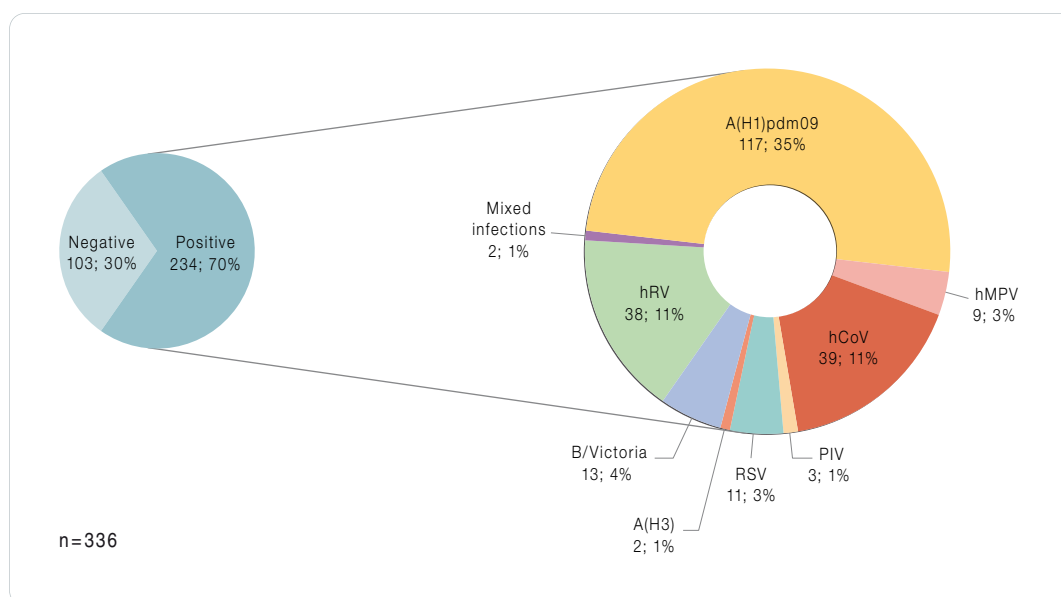


Figure 5 – Virological characterization of ILI cases. Combined laboratory results for influenza A and B, RSV A and B, rhinovirus, parainfluenza, Human coronavirus, adenovirus and Human metapneumovirus.

The 2 isolated influenza A(H3) virus reacted poorly with antibodies, raised against A(H3) reference strains:

A/Hong Kong/5738/2014,

A/Hong Kong/4801/2014 and

A/Switzerland/9715293/2012 (the last one included in 2015/2016 flu vaccine composition).

Integrating virological data in influenza VE

During 2015/2016 influenza season Portugal joined the pilot study that aimed to integrate the virological data in the interpretation of VE results. The implementation of the study included the selection of a representative sample of specimens to describe the detected viruses, identification of amino acid changes that can influence the effect of vaccines and in the end the measurement of clade-specific VE.

Selection of target influenza virus

In the beginning of the season and influenza virus circulation was identified the targeted the

subtype of interest for virus characterization. The emergence of two new subgroups, 6B.1 and 6B.2 of the clade 6B of A(H1)pdm09 viruses, and the predominant circulation of this subtype bring together the criteria for their choice as the target for genetic characterization.

Random case selection and clades by influenza phase

From each defined phase a random sample cases was selected for genetic characterization. In first phase 24 cases were selected and 15 were successfully sequenced: 5 belonged to clade 6B and 10 belonged to subgroup 6B.1. From second phase, 51 cases were randomly selected and 37 A(H1)pdm09 were sequenced: 6 from 6B clade and 24 from 6B.1 subgroup. In the third phase 42 cases were considered for selection, being 41 sequenced. From these only one belonged to clade 6B and 40 to the subgroup 6B.1 (Figure 7).

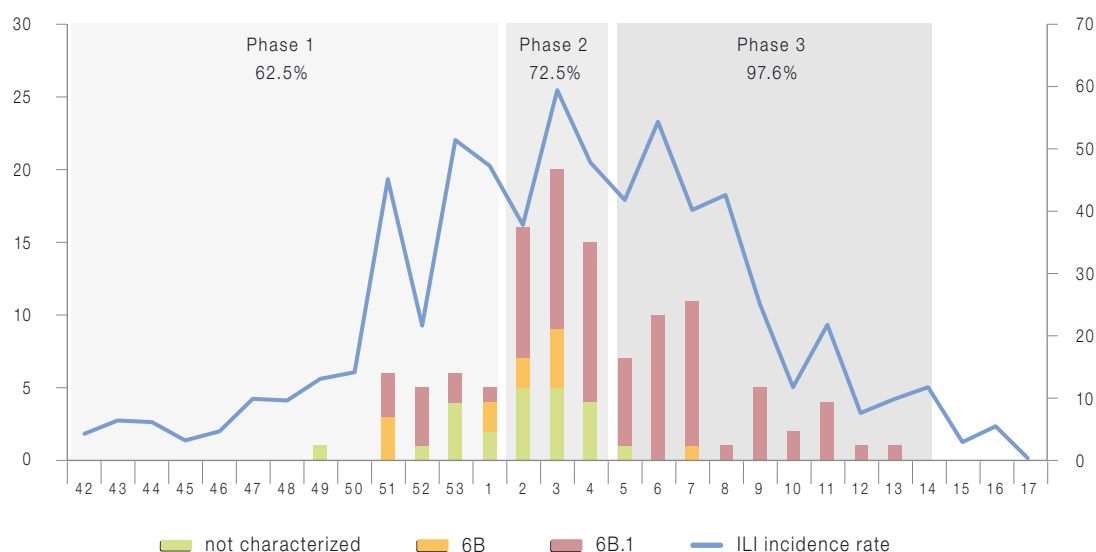


Figure 7 – Distribution of genetic clades and ILI incidence rate per week of symptoms onset; identification of selection phases and respective characterization rates (%).

Performance of genetic characterization

Sequencing was performed directly from the clinical specimen, only when amplification cannot be obtained in the primary sample, sequence was performed in isolated virus (cell culture supernatant), taking into account the amino acid substitutions induced by cell culture adaptation. Samples with different viral load were selected for genetic characterization. A high viral load was considered when the threshold cycle (Ct) of the PCR was under 20, medium viral load for samples with Ct between 20-35 and a low viral load in samples with Ct above 35.

The success of the genetic characterization decreases with increasing Ct value, ranging from 100% of success in samples with Ct < 20 to 66.7% in samples with Ct >35 (Table 2).

Genetic characterization

Following the laboratory protocol for genetic characterizations, genetic analysis based on the HA1 subunit of the hemagglutinin gene was performed on 95 influenza A viruses from

116 selected for characterization (81,9%): 93 H1pdm09 (95%) and 2 H3.

All the 93 influenza A(H1)pdm09 viruses (Figure 8) belong to 6B genetic clade (A/South Africa/3626/2013), however, most of them (n=81, 87%) clustered in the new subclade 6B.1 (A/New York/61/2015). Viruses from this newly emerged subclade are characterized by additional amino acid substitutions: S84N, S162N and I216T. Despite all A(H1)pdm09 viruses are antigenically similar to the vaccine strain, they have already 4 amino acid substitutions located in antigenic sites when comparing to the vaccine strain (Annex III). The relative detection of subclade 6B.1 viruses varied along the season: they represented 41.7% of early season characterized viruses, 62.0% in the peak season and 95.2% in the late season. The two influenza viruses from H3 subtype clustered into the genetic subclade 3C.3a (A/Switzerland/9715293/2013) with the following amino acid substitutions: A138S, F159S and N225D (data not shown).

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

Influenza A(H1)pdm09 virus genetically characterized	n	Medium Ct value	Ct value			
			<20	20-29	30-35	>35
	93	27	6	60	23	4
Total selected viruses	116		6	73	31	6
A(H1)pdm09 characterized/Total selected viruses	(93/116) 80.2%		(6/6) 100 %	(60/73) 82%	(23/31) 74%	(4/6) 67%

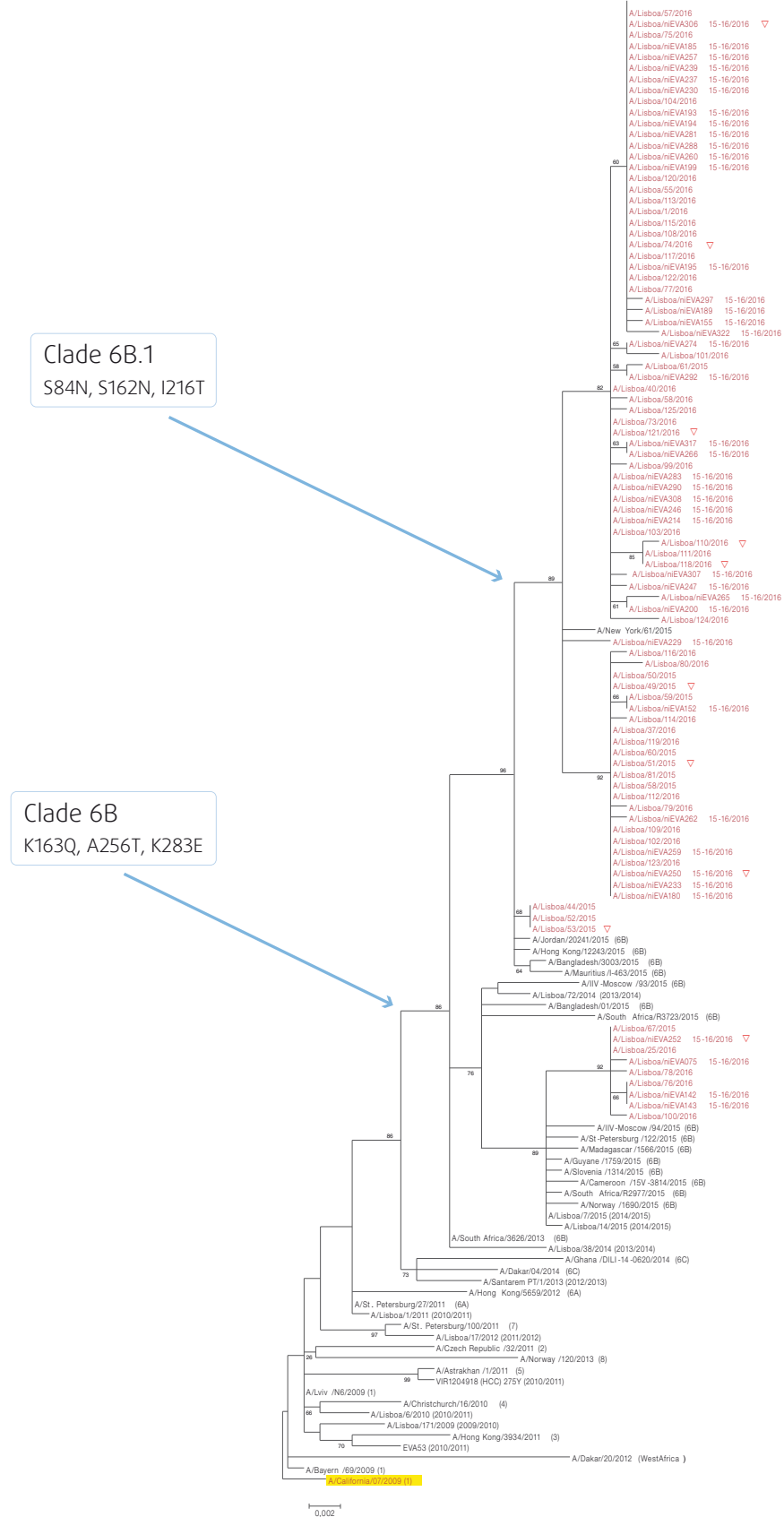


Figure 8 - Phylogenetic tree of influenza A(H1)pdm09 viruses based on the HA1 subunit. Bootstrapp values above 50 are shown. Viruses detected and characterized in this study are shown in red. Reference strains are in black with respective genetic groups in brackets. The 2015/2016 northern hemisphere vaccine strain is highlighted in yellow. ▽ - viruses detected in vaccinated individuals.

Description of participants

A total of 336 ILI patients were selected by the participating GP from week 48/2015 to week 17/2016. The data set for analysis comprised 310 ILI cases. The flowchart of data inclusion/exclusion is presented in [Figure 9](#).

Description of cases and controls

Considering the final sample for statistical analysis (310 ILI patients), 124 ILI patients tested positive for influenza (89.5% were positive for subtype A(H1)pdm2009, 8.9% for B/Victoria and the remaining were positive for A(H3)). The control group consisted in 186 influenza negative ILI patients and was statistically different ($p < 0.05$) from cases in the following ([Table 3](#)):

- **Time between onset and swab:** cases were more prone to be swabbed within 3 days after symptoms onset;
- **Sex:** cases were more frequently men than controls (46.8% vs 28%);
- **Seasonal vaccine in 2015/2016:** controls were more often vaccinated against influenza in the last season than cases (28.4% vs. 11.4%);
- **Any chronic disease:** the prevalence of at least one chronic condition relevant for influenza vaccination was higher in controls (41.6 % vs 28.2%);
- **GP consultations last 12 month:** controls had more GP consultation in the previous year before disease onset than cases (median 3 in controls vs 2 in cases).

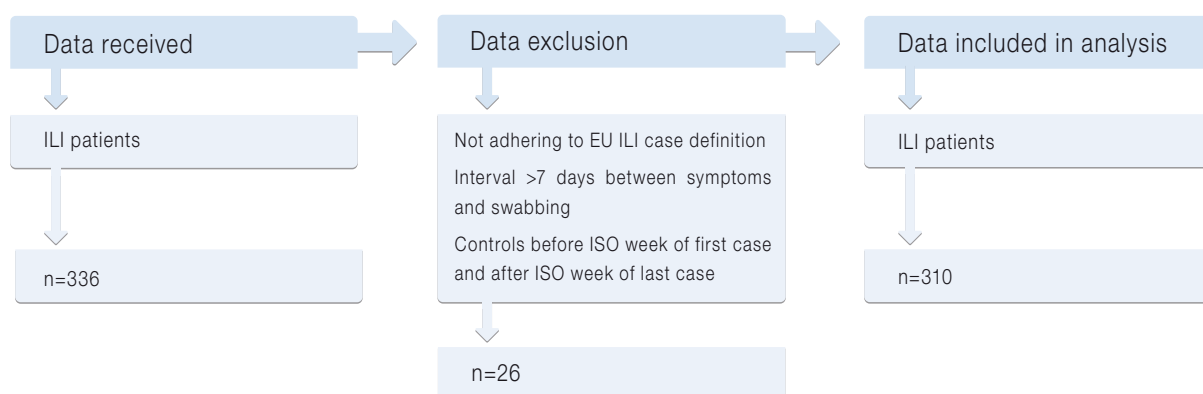


Figure 9 – Flowchart of data inclusion/exclusion.

Table 3 – Description of Cases and Control, from week 49 to week 17 during the 2015/2016 EuroEVA season.

	Cases	Controls
Time between onset and swab collection (days)		
median	2 (124)	2 (186)
$p1^a$	0.0043	
less than 72h,%	91.94 (124)	81.18 (186)
$p2^b$	0.008	
Age, mean	45 (124)	47 (186)
$p1^a$	0.446	
<5		
5-14 years, %	3.2	4.8
15-60 years, %	78.2	66.1
≥60 years, %	18.6	29.0
$p1^b$	0.071	
Sex, male %	46.8 (124)	28 (186)
$p1^b$	0.001	
Smokers, %	12.1 (124)	17.2 (186)
$p1^b$	0.219	
Seasonal vaccine 2015-16, %	11.4 (123)	41 (184)
$p1^b$	0.015	
Chronic diseases (any), %	28.2 (124)	41.9 (186)
$p1^b$	0.014	
Help for bathing, %	1.6 (123)	1.1 (183)
$p1^c$	1.000	
Belongs GP patient list, %	70.2 (124)	135 (186)
$p1^b$	0.643	
Vaccination target group, %	100 (124)	50.5 (186)
$p1^b$	0.077	
GP consultations last 12 mo, median	2 (307)	3 (183)
$p1^a$	0.004	
Any hospitalisations, %	0 (123)	0 (186)
$p1^a$	0.631	
Years of education, median	9 (123)	9 (176)
$p1^a$	0.166	
Co-habitants, median	2 (124)	2 (185)
$p2^a$	0.610	
Statin 30 days before seasonal vaccine, %	16.8 (107)	38.0 (169)
$p2^b$	0.254	

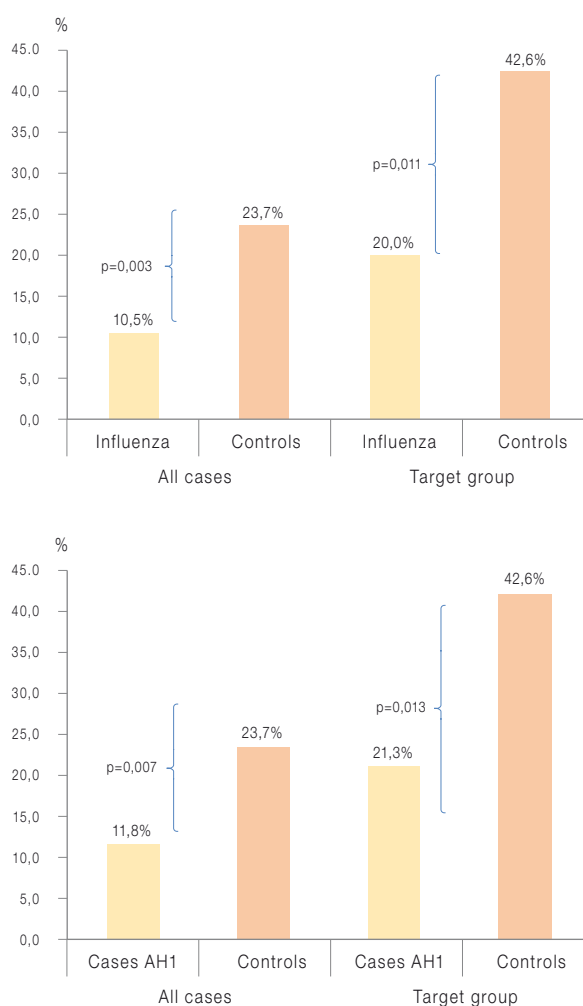
a: Mann-Whitney test; b: Chi-square test; c: Fisher' exact test

Vaccine coverage

Considering all age individuals, vaccine coverage (VC) in controls was 23.7%, statistically higher ($p<0.003$) than in cases (VC=10.5%). Similar results were obtained for the target sub-group for vaccination by the National Health Authorities (VC cases=20.0% and VC controls=42.6%, $p=0.011$).

Restricting the analysis to sub-type AH1pdm09 virus, VC was statistically ($p=0.007$) higher in controls than in cases (23.7% vs 11.7% in all individuals and 42.6% vs 21.3% in the target group).

Figure 10 – Vaccine coverage in cases (all cases and A(H)1pdm09 positive cases) and controls.



Vaccine effectiveness

For 2015/2016 trivalent influenza vaccine, VE estimates was 62.2% (95% CI: 24.1- 82.2%) in all population and 69.9% (95% CI: (27.5%; 87.7%) in the target group for vaccination (Table 4).

It was considered as a confounder all variables which OR, compared to crude OR, changed more than 10 % after M-H adjustment (Figure 11). Age, chronic disease (at least one relevant for the vaccine uptake), number GP consultations in the last 12 months, number of cohabitants, educational group and sex were selected as confounders.

Considering age stratification, VE was 51.7% (95% CI: 16.6- 82.2) for individuals under 65 years old and 79% (95% CI: 4.4- 98.5) for 65 and more years old (Table 5).

Finally, looking at the VE by genetic clades (Table 6), crude estimates indicate that the seasonal vaccine was higher against the new clade (6B.1) then considering 6B group. No adjusted VE estimates were computed due to low sample size.

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	62.2%	(24.1; 82.2)	56.1%	(3.5; 80.0)
AH1pdm09	57.2%	(13.6; 79.9)	54.0%	(-1.5; 79.2)
Target group for vaccination				
Influenza	66.3%	(20.5; 86.5)	65.8%	(12.5; 86.7)
AH1pdm09	63.5%	(13.5; 85.5)	63.9%	(7.7; 85.9)

Table 5 - Vaccine effectiveness (crude and adjusted) by age groups.

	Crude		Adjusted*	
	VE	CI95%	VE	CI95%
< 65 years				
Influenza	51.7%	(-25.9; 83.4)	59.0%	(-9.6; 84.7)
AH1pdm09	44.7%	(85.5; 81.1)	56.2%	(-17.5; 83.7)
≥ 65 years				
AH1pdm09**	79.0%	(4.4; 98.5)	74.9%	(-37.1; 95.4)

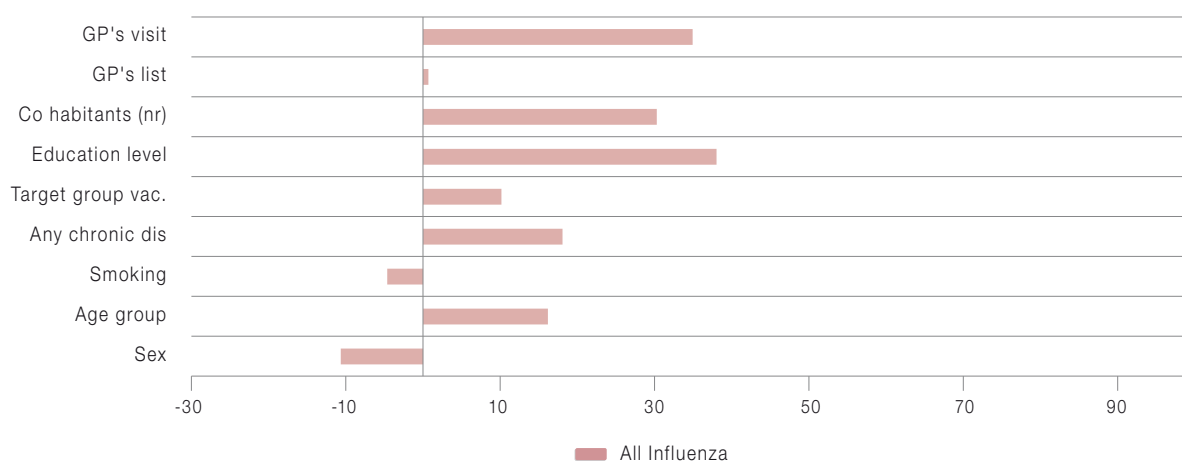
*Adjusted for chronic disease (at least two relevant for the vaccine uptake), number of GP consultations in the last 12 mo, number of cohabitants, educational group and sex;

**all cases were positive for AH1pdm09.

Table 5 - Crude vaccine effectiveness by genetic clade.

	Cases 6B.1		Cases 6B	
	VE	CI95%	VE	CI95%
	67.3%	(21.4; 88.1)	35.5%	(-220.5; 93.4)

Figure 11 – Seasonal vaccine 2015/2016 Odds Ratio change (%) after adjustment for potential confounding factors



5

Discussion and conclusions

5.1 Influenza vaccine effectiveness in the 2015/2016 season

Overall adjusted VE point estimates indicate that this season, the influenza vaccine conferred low/moderate protection against all influenza viruses (around 56%). This value did not change when restricting to vaccine protection against A(H1N1)pdm09 (54.0%) and one possible explanation may be that this virus subtype was dominant in the majority of the season (from week 53/2015 until week 8/2016 it was the only detected subtype of virus).

Although the emergence of new genetic subgroups of the 6B clade, which shows genetic evolution of this subtype, all A(H1N1)pdm09 viruses remained antigenically similar to the 2015/2016 vaccine strain (A/California/7/2009).

Looking in to the effect of the vaccine against A(H1N1)pdm09 in the target group for vaccination, adjusted VE increased to 64% (approximately 66% for all influenza virus).

Age stratification indicates that the vaccine was more effective in the elderly, reaching an adjusted point estimate of approximately 75% in the elderly with 65 and more years. Nevertheless, this result should be interpreted with caution given the low precision of the estimates (95%CI varied between -37% and 95%).

These overall EuroEVA estimates are in the same range of the intermediate results published in United Kingdom [49.1% against A(H1N1)pdm09] ⁹ and by the I-MOVE group related to pool anal-

ysis of 10 European countries (46.3% against all influenza) ¹⁰. Regarding to age group estimate, similar results were reported in Canada [56% for adults between 20 and 64 years-old against medically attended, laboratory-confirmed A(H1N1)pdm09] ¹¹. However, for individuals aged above 65, VE estimate in Denmark indicates a low protection of the trivalent vaccine against influenza A(H1N1)pdm09 (around 35%) ¹², result quite dissimilar from the EuroEVA estimates.

Effects of adjustment

In order to obtain adjusted VE point estimates, potential confounders were analyzed and selected if they change more than 10% the crude seasonal vaccine OR. This season, several confounders were identified, namely, age, chronic disease (at least one relevant for the vaccine uptake), number of GP consultations in the last 12 months, number of cohabitants, educational group (*all positive confounders*) and sex (*negative confounder*). These set of confounders had an impact in the final estimates, by reducing the adjusted VE point estimates and reducing the precision (increasing the random error).

They were also used to adjust age stratified VE, by changing the variable at least one chronic condition to at least two conditions in the 65 plus group in accordance with "Protocol for hospital-based test negative case control studies to measure seasonal influenza vaccine effectiveness against influenza laboratory confirmed SARI hospitalisation among the elderly across the European Union and European Economic Area Member States" ¹³.

When looking to the comparison of cases and controls characteristics, one variable highlights given their potential classification error of cases or controls: time between onset and swab. According to the results, cases were more prone to be selected within 3 days of symptoms onset and as a consequence some controls may be classified as false negative cases. However, the real time RT-PCR used in EuroEVA allows a good detection even with low viral load, thus reducing the false negatives¹⁴ and ensures that classification of cases and controls was correct.

5.2 Logistical aspects of EuroEVA 2015/2016 implementation

Participation rate

The number of GP that participated in EuroEVA increased during this season (84 vs 50 in 2014/2015) and this reflected in the number of effective GP participants (51 vs 31 in 2014/2015) and in ILI patients enrolled in the study (336 vs 268 in 2014/2015). Also, their geographical distribution is more representative of Portugal, given that includes not only mainland but also the autonomous islands. The improvement of these process indicators may be related to the reinforcement of resources allocated to the project and to communication strategies that included close e-mail/ telephone contact but also the dissemination of a newsletter of the project, in a monthly basis.

ILI cases selection

As in the previous studies, the EU ILI definition was used for the identification of ILI cases. From all the ILI cases enrolled with complete biological and clinical information, only 22 out of 336 did not meet the EU ILI case definition. This result indicates that a correct ILI case selection has been maintained in the EuroEVA study. In part, this may be due to the long experience of most GPs in participating in the EuroEVA study and to intensive training of the new GP's that joined the project for the 1st time.

A systematic ILI case selection scheme was again used this season, consisting of randomly attributing a different day of week to each GP (from Monday to Thursday) in which they would start selecting ILI cases, keeping the restriction of selecting only two ILI cases per week from individuals with less than 60 years of age, and no limit to the number of 60+ ILI cases selected. This strategy was intended to increase the previously observed low proportion of ILI cases with 65+ years of age, but proved relatively low successful in practice.

Looking into the number of recruited cases per GP and week of symptoms onset, we verify that the majority of GP fulfilled with this sampling procedure (only 5 out of 51 participants occasionally recruited more than 2 ILI cases with less than 60 years). For these GP's, the training for the systematic selection will be reinforced next season.

5.3 Integrating virological data in influenza ve studies: pilot study

This season, Portugal along with other 6 countries piloted the integration of virological data in the VE study. Since the beginning, in Portugal, the selection of virus type and subtype to be targeted in the pilot study was straightforward given the dominance of virus A, subtype A(H1)pdm09. Nevertheless, the team considers that in future seasons, if there is a co-predominance of different influenza types/subtypes, the selection of a target virus for genetic characterization could impose some difficulties. Also, the selection of the sampling fraction to be adopted throughout the study was challenging in the beginning of the season. For this reason, we propose to increase the sample fraction in the 1st phase extraction, according to the laboratory capacity, and continue to incorporate data on description of influenza virus circulation from external sources, namely from Tessy.

At the end of the season, we were able to successfully characterize 93 out of 116 A(H1)pdm09 cases, which corresponds to a characterization rate of approximately 80%. This effort reflected not only on more virological data that could be used to explain current VE estimates, given the random selection of cases, but also reinforced the availability of genetic sequences in GISAID database in real time and not at the end of the season. In order to be able to respond to this challenge, the team had to re-organize to be

succeeded in the characterizations and this increased the workload. Nevertheless, given the good flow of communication between epidemiology group and laboratory group, the pilot study was feasible and with good results.

6

Key messages

- The 2015/2016 influenza vaccine conferred low/moderate protection against the main circulating influenza virus subtype, A(H1)pdm09;
- VE against influenza A(H1)pdm09, subclade 6B.1 were similar to VE against all A(H1)pdm09, this fact is in line with the antigenic similarity between all detected A(H1)pdm09 viruses. Meanwhile it was observed genetic evolution of this subtype, with appearance of new subclades in circulation, 6B.1 and 6B.2;
- The pilot study to assess the feasibility of integration of genetic results in VE study was well succeeded with a characterization rate of 80% and for future seasons the increase of gene sequencing using NGS is being evaluated;
- The reinforcement of resources allowed the increase of participating GP and final sample size.

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ANNEXES

Annex I – EuroEVA 2015/2016 questionnaire

Nome: _____

N.º Cartão de utente/ Proc. Clínico: _____

Código do caso _____

(Destacar por aqui)

6632305308 Efectividade da Vacina Antigripal EuroEVA - 2015/2016

Código do caso 019019 - _____

Código Médico EuroEVA EVA <u>019019</u>	A preencher pelo INSA N.º EVA _____ Req _____ Data _____/_____/_____ Amostra ZNF <input type="checkbox"/> Outro <input type="checkbox"/> Técnico <input type="checkbox"/> <u>Pesquisa de vírus</u> Negativo <input type="checkbox"/> { AH1pdm09 <input type="checkbox"/> AH3 <input type="checkbox"/> Positivo <input type="checkbox"/> { B/Vic <input type="checkbox"/> B/Yam <input type="checkbox"/> B <input type="checkbox"/>
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Unidade de Saúde USF Beira - Douro Polo
Rio Mau
Médico: Pedro Roque

Data de Colheita _____/_____/_____
(dd / mm / aaaa)

Informação Relativa ao Doente
Sexo Masculino Feminino Idade _____ anos _____ meses

Síndrome Gripal
Data de início dos sintomas _____/_____/_____
(dd / mm / aaaa)

Início súbito (<24h)	S	N	D
Febre ou febrícula _____°C	S	N	D
Mal-estar geral, debilidade, prostração	S	N	D
Cefaleia	S	N	D
Mialgias, dores generalizadas	S	N	D
Tosse	S	N	D
Dor de garganta, inflamação da mucosa nasal e faríngea, sem sinais respiratórios relevantes	S	N	D
Dificuldade respiratória	S	N	D
Calafrios/arrepios	S	N	D
Contacto com doente com gripe	S	N	D

Tomou antivirais durante os últimos 14 dias?
 Não
 Sim, o doente
 Sim, um co-habitante

Nome do antiviral
 Oseltamivir (Tamiflu)
 Zanamivir (Relenza)
 Outro

Vai ser tratado com antiviral
 S N

Nome do antiviral _____

Colar na zaragatoa

Legenda: S – Sim N – Não D - Desconhece

6114305303 Questionário Síndrome Gripal (Confirmar as respostas diretamente com o(a) doente)

O doente não pertence à minha lista Código do caso 019019 - _____

Nesta época (2015/2016), o(a) doente foi vacinado(a) contra a gripe?	S	N	D	
Se sim, vacinado em (Se não sabe a data exacta, indique a mais aproximada)	_____/_____/_____/2011			
Qual era o nome da vacina?	Não sabe <input type="checkbox"/>			
O doente tomava estatinas 30 dias antes da toma da vacina ?	S	N	D	
O doente foi vacinado contra a gripe sazonal na época 2014/2015?	S	N	D	
O doente é profissional de saúde ou cuidador num lar/casa de idosos	S	N	D	
O(a) doente é co-habitante ou cuidador de crianças que tenham risco elevado de desenvolver complicações, cuja idade é < 6 meses	S	N	D	
A doente encontra-se grávida?	S	N	D	NA
Se sim em que trimestre se encontra?	____ Não sabe <input type="checkbox"/>			
História tabágica do(a) doente:	F	ExF	NF	D

F (Fumador) ExF (Deixou de fumar há mais de um ano) NF (Nunca fumou) D (Desconhece)

Doenças crónicas

Diabetes	S	N	D	
Doenças cardiovasculares (cardiopatía congénita, hipertensão, isquémica e insuficiência cardíaca crónica)	S	N	D	
Doença renal crónica (insuficiência renal crónica, síndrome nefrótica)	S	N	D	
Doença hepática crónica (cirrose, atresia biliar, hepatite crónica)	S	N	D	
Obesidade (IMC >=30)	S	N	D	
Doença respiratória crónica (asma, bronquite crónica, enfisema, fibrose quística, pneumoconioses, displasia broncopulmonar, fibrose pulmonar)	S	N	D	
Imunodeficiência congénita ou adquirida	S	N	D	
Doença neuromuscular com compromisso da função respiratória	S	N	D	
Nos últimos 12 meses, quantas vezes foi o(a) doente hospitalizado devido a uma destas doenças crónicas?	____ Não sabe <input type="checkbox"/>			
Número de consultas de Medicina Geral e Familiar, nos últimos 12 meses?	____ Não sabe <input type="checkbox"/>			
Quantos anos de escolaridade o doente completou com aproveitamento?	____ Não sabe <input type="checkbox"/>			
Quantas pessoas vivem na mesma casa com o(a) doente? (familiares ou não familiares, sem contar com o doente)	____ Não sabe <input type="checkbox"/>			
O(a) doente necessita de ajuda para tomar banho? (aplicável se tiver 10 ou mais anos de idade)	S	N	D	NA

Legenda: S - Sim N - Não D - Desconhece NA- Não aplicável

Annex II – List of variables, definitions and coding

Variable name	Type	Values and coding	Definition
idcountry	Numeric	Coded according to international country codes	Identifier uniquely identifying the country
participate	Numeric (binary)	0 = No 1 = Yes	Agrees to participate
refuse	Text	Unique integer	Reasons for refusal to participate
Id	Numeric (continuous)	0 = control 1 = case	Unique number for each record
Case	Numeric (binary)	Unique integer	Identifies cases and controls
gpcode	Numeric (continuous)	0 = No 1 = Yes	Unique number for each GP (preventing identification of GP)
gp_patient_list	Numeric (categorical)	Integer	Patient belongs to GP patient list
age	Numeric (continuous)	0 = female 1 = male	Age of each participant in years
sex	Numeric (binary)	dd/mm/yyyy	Sex of study participant
onsetdate	Date	dd/mm/yyyy	Date of onset of symptoms
swabdate	Date	0 = No 1 = Yes	Swabbing date
fever	Numeric (categorical)	8 = Do not know 0 = No 1 = Yes	Fever
malaise	Numeric (categorical)	8 = Do not know 0 = No 1 = Yes 8 = Do not know	Malaise
myalgia	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Myalgia
cough	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Cough
sorethroat	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sore throat
suddenonset	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Sudden onset
headache	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Headache

Variable name	Type	Values and coding	Definition
shortness of breath	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Weakness
lab_res	Numeric (categorical)	0 = Negative 1 = Positive 8 = Do not know	Laboratory result (positive/negative)
lab_virus	Numeric (categorical)	0 = B 1 = AH3 2 = AH1pdm09 3 = B/Vic 4 = B/Yam	Laboratory result: virus type
lab_subtype	Text		Laboratory result: virus subtype
seasvaccany	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Received seasonal influenza vaccine 2011-12
seasvacdate	Date	dd/mm/yyyy	Vaccination (seasonal vaccine) date
seasvacctype	Text		Type of seasonal vaccine (brand name)
vac_13	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Previous seasonal influenza vaccination 2013-14
health_prof	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	The patient is a health professional or a care taker
Statin	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	The patient took statin 30 days before the vaccine uptake
cohab_risk	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	The patient is a co-habitant of a risk patient with less than 6 months
pregn	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know 7 = Not applicable (if sex=1 or sex=0 and age>=50 and age<=15)	Pregnancy
preg_trim	Numeric	1, 2, 3; 9=do not know 7 = Not applicable if pregn=7	Pregnancy trimester
smoking	Numeric (categorical)	0 = Never 1 = Former 2 = Current 8 = Do not know 7 = Not applicable if age<15 years.	Never, former (stopped smoking at least 1 year before inclusion in the study), current smoker
diabetes	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Diabetes and endocrine

Variable name	Type	Values and coding	Definition
Heart disease	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Congenital heart disease, hypertensive heart disease, ischemic heart disease, chronic heart failure
Chro_Renal_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Chronic renal disease
Chro_Hepatic_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Chronic hepatic disease includes: cirrhosis, biliar atresia and chronic hepatitis
obesity	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	IMC>=30
Chro_Resp_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Chronic respiratory disease includes: asthma, chronic bronchitis, emphysema, brochopulmonary dysplasia, cystic fibrosis, pneumoconiosis and pulmonary fibrosis
Immuno	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Immunodeficiency congenital or acquired: conditions that suppress the immune function due to underlying disease and/or therapy, e.g. chemotherapy, HIV infection
NeuMusc_dis	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Neuromuscular disease with compromised respiratory function
severity	Numeric (count)	integer 998 = Do not know	Number of hospitalisations previous year for the chronic disease
gpvisit	Numeric (count)	Integer 98 = Do not know	Number of GP consultations previous year
educa_years	Numeric (count)	Integer 98 = Do not know	Number of completed education years
co_habitants	Numeric (categorical)	integer 98 = Do not know	Number of co-habitants in the household
fs_bath	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know 7 = Not applicable if age<10 years.	Requires assistance to bath
antivir	Text	0 = No 1 = Yes 8 = Do not know	Administration of antivirals
antivirtype	Numeric (categorical)		Type of antiviral (brand name)
res_home	Numeric (categorical)	0 = No 1 = Yes 8 = Do not know	Exclusion criteria: living in a residential home
contra		0 = No 1 = Yes 8 = Do not know	Exclusion criteria: contraindication for influenza vaccination

_Department of Epidemiology

National Health Institute Dr. Ricardo Jorge
Av. Padre Cruz, 1649-016 Lisbon, Portugal
Tel.: (+351) 217 526 404
Fax: (+351) 217 526 499
E-mail: dep@insa.min-saude.pt

_Infectious Diseases Department

National Health Institute Dr. Ricardo Jorge
Av. Padre Cruz, 1649-016 Lisbon, Portugal
Tel.: (+351) 217 519 200
Fax: (+351) 217 526 400
E-mail: ddi@insa.min-saude.pt

Centre of Public Health Dr. Gonçalves Ferreira
Rua Alexandre Herculano, n.321 4000-055 Porto, Portugal
Tel.: (+351) 223 401 100
Fax: (+351) 223 401 109
E-mail: inforporto@insa.min-saude.pt

Centre of Study and Vectors and Infectious Diseases
Dr. Francisco Cambournac
Av. da Liberdade, n.5, 2965-575 Águas de Moura, Portugal
Tel.: (+351) 265 938 295
Fax: (+351) 223 401 290
E-mail: cevdi@insa.min-saude.pt