Case control study for measuring influenza vaccine effectiveness in Portugal - Final report
Season 2009-10

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25 June 2010
Lisbon - Portugal
This report was prepared for the Project “Monitoring Influenza vaccine effectiveness during influenza seasons and pandemics in the European Union” and describes the results of the pilot case control study, conducted in Portugal under the Protocol Agreement celebrated between EpiConcept SARL, Paris and Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, signed on August 12th 2009, with an addenda signed on December 17th 2009.
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Summary

Introduction

Influenza epidemics have substantial consequences on human health by increasing mortality and morbidity rates. For both seasonal and pandemic influenza, the vaccination is the main method for preventing the disease and its more severe complications. Thus, timely evaluation of the vaccine effectiveness is of major importance for public health decisions. Since 2008-2009, Portugal has been participating in I-MOVE project that aims to estimate seasonal and pandemic vaccine effectiveness during and after the influenza season.

In 2009-2010 Portugal has joined the I-MOVE multi-center case control study together with Spain, Ireland, France, Italy, Hungary and Romania, using a common protocol and with the objective of estimate the seasonal and pandemic influenza vaccine effectiveness respectively in the elderly (65+) and in all age groups.

Material and Methods

The general design was a case-control approach where laboratory confirmed influenza cases (ILI+) were compared to laboratory negative influenza ILI patients (ILI-).

Cases and controls were identified among patients that presented influenza-like illness to a participating GP. The study was designed to use preferably routine data provided by the Portuguese system of integrated clinical and virological influenza surveillance, based on the Médicos Sentinela (MS) network. In this context the ILI patients were selected systematically (four per week per GP) using the EU ILI case definition. Data on confounding factors and effect modifiers was collected using a standardized questionnaire. An ILI patient was considered vaccinated if he/she had received one dose of the vaccine at least 14 days prior onset of symptoms.

Data collection was set from week 47 of 2009 up to week 14 of 2010, although data was analyzed only until week 7 (week of the last laboratory-confirmed case).

Vaccine effectiveness was estimated as one minus the odds ratio of being vaccinated in cases versus controls adjusted for confounders by logistic regression.

Results

During this season the influenza activity in Portugal was associated with the predominant circulation of the pandemic influenza virus A(H1N1)v, and during the study no seasonal influenza virus was detected. From the 53 GP that accepted to participate in the study, 32 (60%) effectively participated in the study by selecting patients, collecting swabs and data.

For the seasonal vaccine study (patients with 65+ years of age) 63 patients were included, 10 of which
did not meet the inclusion criteria. From the 53 ILI cases considered, none was positive for seasonal influenza virus, 4 were positive for A(H1N1)v and 49 were negative.

The final sample size of the pandemic vaccine study consisted on 244 ILI patients, 56 of which were excluded from the analysis for not meeting the inclusion criteria. Among the remaining 188 patients, 32 (17.0%) were A(H1N1)v positive, and the rest were influenza negative.

When comparing cases and controls the following significant differences were identified:

1. Cases were younger than controls (mean age Ca: 36yrs vs Co: 46yrs);
2. Controls presented a higher prevalence of cardiovascular diseases (Ca: 6.3% vs Co: 29.0%);
3. Cases presented a higher prevalence of chronic renal disease (Ca: 6.3% vs Co: 0.0%);
4. Controls presented a higher percentage of patients that belonged to the GP list (Ca: 31.3% vs Co: 52.6%);
5. Controls presented a higher median number of visits to the GP over the last 12 months (Ca: 3 vs Co: 4);
6. Cases presented a higher median number of co-habitants (Ca: 3 vs Co: 2);
7. Cases had fever in a higher percentage (Ca: 96.8% vs Co: 78.7%)

Concerning the primary objective of the study:

1. Pandemic vaccine coverage was higher in controls than in cases - Ca: 0.0% (0/32) vs Co: 6.4% (10/156) – but not statistically significant;
2. Seasonal vaccine coverage was higher in controls than in cases – Ca: 25.0% (8/32) vs 33.3% (52/156) – but not statistically significant.

The pandemic vaccine effectiveness was not computed given that none of the cases was vaccinated.

Since none of the ILI cases was positive for seasonal influenza virus, no seasonal vaccine effectiveness was computed.

On the other hand, the effect of the seasonal vaccine on the A(H1N1)v infection was not statistically significant. The crude estimate of seasonal vaccine effectiveness on the A(H1N1)v infection was 33% CI95%[-59;72] and after adjustment for possible confounders (group, sex, month of ILI onset, chronic diseases, GP visits in last 12mo, patient belongs to GP list and number of co-habitants) was 29% CI95%[-254;86].

**Discussion**

The current study was unable to provide pandemic and seasonal influenza vaccine effectiveness estimates at national level. This was mainly due to the low number of influenza laboratory confirmed cases enrolled and to the fact that none of the cases was vaccinated for the pandemic strain.

The factors that contributed the most for this result were:
1. Late definition of the pandemic vaccination campaign, namely the start and target groups, thus postponing the beginning of the study to week 50;
2. The start of pandemic vaccination campaign (week 44) and of the pandemic vaccine study (week 50) during the epidemic period (week 44 to week 51);
3. Low pandemic vaccine coverage (due to late vaccine campaign) - no vaccine failures;
4. Only 60% of effective GP participation;
5. No ILI cases positive for seasonal influenza virus - impossible to estimate seasonal VE.

Conclusions

The current study was unable to provide pandemic and seasonal influenza vaccine effectiveness estimates at the national level.

This situation has also occurred in other I-MOVE site studies; nevertheless the joint effort of the multi-centre sites has enabled the estimation of a pandemic VE with a reasonable precision.

Another objective that was added to the study protocol was the effect of 2009-2010 seasonal vaccine on the A(H1N1)v infection. The results of this study were unable to find any association between 2009-2010 seasonal vaccine with the A(H1N1)v infection.

Regarding study design and logistic aspects, the 2009-2010 Euroeva study has succeeded in the introduction of the EU ILI definition, of the systematic selection of ILI patients with weekly SMS reminders; in increasing the participation rate and sample size and increasing the representativeness of general ILI cases population by the inclusion of the ILI cases out of the GP patient list.

Recommendations:

The main recommendations focused on;

- To increase sample size, mainly in the elderly population (aged 65 years or more);
- To increase the total number of participating GP’s in the study by exploring other sources of GP’s recruitment;
- To register if the enrolled ILI cases belongs to the influenza vaccine recommended target groups at the national level;
- To start the study as soon as possible taking into consideration the beginning and evolution of the vaccination campaign;

Finally we also recommend continuing the harmonization of the study designs between participating countries assuming has a goal the multi-centre study.
Introduction

Every year, influenza epidemics have substantial consequences on human health by increasing mortality and morbidity rates. Particularly, during a pandemic season, albeit occurring rarely, can have major impact on younger individuals. For both seasonal and pandemic influenza, the vaccine is the main method for preventing the disease and its more severe complications. The evaluation, in the same season, of the vaccine effectiveness is of major importance for public health decisions, especially since the vaccine is reformulated every year.

In this context, the National Institute of Health (INSA) in Portugal, conducted during the 2005/2006 and 2006/2007 seasons, two pilot studies with a cohort design. 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.

The European Centre for Disease Prevention and Control (ECDC) launched in 2008 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 Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), through its Departments of Epidemiology and Infectious Diseases, was invited by the leader of the winning Consortium (EpiConcept,SARL) to participate in the project Monitoring influenza vaccine effectiveness during influenza seasons and pandemics in the European Union (I-MOVE). INSA had previously participated in EUROEVA, a pilot study conducted to test a 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. The study was designed to use preferably routine data provided by the Portuguese system of integrated clinical and virology influenza surveillance, based on the GP sentinel network - Médicos Sentinela (MS).

The network of “Médicos-Sentinela” has been operating since 1991 and is constituted by approximately 150 GP that participate in a voluntary way. The MS population under observation covers 1.2% of the Portuguese population and is representative by sex and age. During the influenza period (from week 40-20) the criteria for the incidence rate estimates have been based on the presence of 6 or more symptoms from the set of 8 symptoms associated with ILI according to the International Classification of Health Problems in Primary Care (ICHPPC-2): sudden onset, cough, chills, fever, weakness or exhaustion, body aches, sore throat with no relevant inflammatory signals and contact with patient with influenza. The virological surveillance consists on laboratory analysis of nose and throat swabs of all patients with ILI that GPs are asked to take. On average the network yields about 10 samples per week for virological determination.

In July 2009, Portugal was chosen to participate in the I-MOVE project for the present influenza season (2009-2010), with a case-control design (Euroeva 2009) together with Spain, Ireland, France, Italy,
Hungary and Romania. Meanwhile, in the context of the ongoing Pandemic (H1N1)2009 and the development of the new vaccine against the A(H1N1)v influenza virus, the Euroeva 2009 study protocol was revised in October 2009 in order to be adapted to the new situation and to include the objective of estimating the A(H1N1)v monovalent vaccine effectiveness together with the objective of estimating the seasonal vaccine effectiveness.

In Portugal the Preparedness Plan for the Pandemic, which was activated on April 2009, programmed the vaccination of about 30% of the Portuguese population, aiming at protecting not only the most vulnerable citizens, like pregnant women and chronically ill patients, but also to protect those in charge of essentials sectors of society. The vaccine selected to be administered in Portugal was the Pandemrix® vaccine, from the pharmaceutical company Glaxo Smith Kline (GSK). The vaccination campaign, coordinated by the Directorate General of Health, began on October 26, and vaccines were administrated mainly at health centres, but also at hospitals and other services (police departments, several companies interested in administrating vaccines to its personnel, etc.). For vaccination, persons should present a statement from a medical doctor certifying its inclusion in one of the three target group (A, B or C), but the decision of being vaccinated belonged to the individual. To facilitate access to the vaccine, patients with diabetes, pregnant women and children (when vaccination has included healthy children) did not need to present the statement.

Seasonal vaccination campaign started on week 37 and several different brands were available at pharmacies to general public. Target groups for the seasonal vaccine included the elderly (with 65 years and above), chronically ill patients, persons with imunodepression and health professionals.

This report describes the project development and the results obtained from the data collected from week 47 to week 14.
**Objectives**

**Primary objective**

The primary objectives were to measure:
- pandemic influenza vaccine effectiveness in the general population;
- seasonal influenza vaccine effectiveness among people aged 65 years and above.

**Secondary objectives**

To estimate pandemic and seasonal VE:
- in each of the participating countries;
- by risk groups.

Seasonal vaccine:
- to estimate VE on pandemic influenza among individuals of all age (supplementary objective)
- to estimate VE by influenza subtype;
- to provide intra-seasonal VE estimates;
- to monitor VE each year.

Pandemic vaccine:
- to provide early VE estimates;
- to estimate VE for one and two doses;
Methods

Study design

The general design was a case-control approach where laboratory confirmed influenza cases were compared to laboratory influenza-negative ILI patients.

Study population and sampling design

The eligible population for the study included all ILI cases, non institutionalized and resident on the participating GPs catchment area.

For the seasonal vaccine, the study population was composed of individuals aged 65 years and above with no contra-indication for influenza vaccination.

For the pandemic vaccines, the study population was composed of individuals from all ages with no contra-indication for vaccination with the pandemic vaccine.

The sampling was performed in two steps:

1. GPs were contacted and selected from a list of sentinel doctors belonging, or that had belonged, to the MS network. All the GPs of the Portuguese Sentinel Network were invited to participate on the EUROEVA 2009-2010, by ordinary mail and e-mail. Those GPs were asked to select others to participate on the study, which were contacted later. All GPs that participated on the EUROEVA 2008-2009 were also invited to participate on the current study (EUROEVA 2009-2010).

2. Each GP that accepted to participate has selected four ILI cases (EU ILI definition) per week (two aged 65+ and two with less than 65 years of age) from their weekly consults (patient could belong or not to the GP list).

Study period

In order to estimate seasonal VE, ILI cases with 65 or more years of age were selected by GPs starting on November 2009 (week 47). For the pandemic VE study, ILI cases from all ages were collected starting on December 2009 (week 50).

Data collection for both studies was finalized in week 14 given that since week 7 none of the ILI cases enrolled in the study were positive for influenza.

The results presented in this report comprise data collected since the above mentioned starting dates and week 14 of the influenza season.
Outcome

A confirmed case of influenza virus infection is defined as a person with an influenza-like illness with laboratory confirmed influenza A (H1N1), A(H1N1)v, A(H3N2) or B virus infection by one or more of the following tests:

1. real-time RT-PCR
2. viral culture

Cases

Case definition

Influenza-positive ILI cases were considered as Cases. A case of influenza like illness (ILI) was defined as an individual who consults a participating GP, presenting a sudden onset of symptoms and at least one of the following four systemic symptoms (EU criteria):

- fever or feverishness;
- malaise;
- headache;
- myalgia;

AND at least one of the following three respiratory symptoms:

- cough;
- sore throat; and
- shortness of breath.

For seasonal VE study, Cases were defined as an ILI case with a respiratory sample positive for any sub-types of seasonal influenza virus, A(H1N1), A(H3N2) and B.

For pandemic VE study, Cases were defined as an ILI case with a respiratory sample positive for A(H1N1)v.

Laboratory confirmation

Specimens collection

The success of virus diagnosis largely depends on the quality of the specimen and the conditions for transport and storage of the specimens before it is processed in the laboratory.

Specimens were collected from ILI cases who consult their GP within 7 days after onset of clinical
symptoms for influenza like illness.

Nasopharyngeal swabs, or a combined nasopharyngeal with oropharyngeal swab were acceptable. The specimens were collected into a suitable transport medium. This procedure was conducted by the GP himself or by a nurse under his supervision.

Each sample was identified 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.

**Storage, transport**

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

**Laboratory Tests (RT-PCR / Culture)**

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 (Atlanta). Selected isolates were sent to the WHO Collaborating Center in London for further study.

The rapid detection and (sub)typing of seasonal influenza viruses was performed by a multiplex “in house” real-time RT-PCR targeted to the haemagglutinin gene of influenza A and B. This is a powerful technique for the identification of influenza virus genomes even when they are present at very low levels.

The CDC real-time RT-PCR protocol for the detection and characterization of the pandemic influenza A(H1N1) virus was performed for the laboratory confirmation of the new influenza A(H1N1) virus infections.

**Strain characterization**

The phylogenetic analyses of the influenza virus isolates was done by sequencing of specific regions of the haemagglutinin (HA1 subunit) and neuraminidase genes (using primer sequences made available by the CDC), for a subset of isolates from the beginning, the peak and the end of the season, representing 25% of the ILI positives, using the Clustal Method on the Megalign programme (DNAStar 99 package, Lasergene).

The reference laboratory follows internal control procedures and external quality control programs organized by Global Influenza Surveillance Network (GISN) and the World Health Organization (WHO).
**Case finding**

**Procedures to select ILI cases**

Cases were identified among patients that presented ILI to a participating GP. For the purpose of estimating seasonal VE, GPs selected ILI cases with 65 years or more and for estimating the pandemic VE, ILI cases of all age were recruited. The ILI case could occur among GPs patient list or not, provided that an encounter patient/GP took place.

To estimate both seasonal and pandemic H1N1 influenza VE, ILI cases were recruited using the EU case definition, respecting the exclusion criteria (described below) and using a systematic sampling method. This systematic sampling procedure consisted on the selection, by each GP, of the first four ILI cases (2 less than 65 years, 2 with 65 years or more) of each week. To avoid bias 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.). In this way, each GP had a different starting day of the week and received a SMS reminder the day before the start of his “week”.

**Case inclusion criteria**

Cases were eligible if they meet the above case definition and accepted to participate. An oral informed consent was requested to ILI cases after explaining the objectives of the study.

**Case exclusion criteria**

For both seasonal and pandemic H1N1 influenza VE, cases were excluded if they:

- refused to participate in the study;
- were not eligible for influenza vaccination;
- were institutionalised;
- were unable to give informed consent or follow an interview in their native language because of aphasia, reduced consciousness, or other reasons.

For seasonal VE, participants were excluded if they did not meet the age criteria (65+). For pandemic VE, cases were excluded if they had previously been diagnosed with influenza A(H1N1)v after the start of the pandemic.

All the excluded cases were registered in an appropriated form.
Control groups

ILI influenza negative controls

For both seasonal and pandemic VE studies, Controls corresponded to individuals that presented ILI to a participating GP but were tested negative for influenza infection. As for Cases, Controls were systematically selected from the GP list or other, provided that an encounter patient/GP took place. The systematic sampling procedure was already described (for Cases).

- For seasonal influenza VE study, ILI cases that tested negative for seasonal influenza A(H1N1), A(H3N2) and B were included in the Control group.
- For pandemic influenza VE study, all ILI cases that tested negative for influenza A(H1N1)v were included in the Control group.

The exclusion criteria described for Cases are also applicable for Controls. Excluded controls were registered in an appropriated form.

Community controls

This group of controls was selected from an already implemented vaccine coverage monitoring survey, conducted every year since 1998\textsuperscript{10}.

Controls were selected from a population-based sample of households with landline telephone. Data was collected via Computer Assisted Telephone Interview during April 2010. Information with interest for the current study comprised the vaccine status (seasonal and Pandemic H1N1 2009 - only for individuals with 18 or more years of age), influenza-like illness symptoms manifested from September to the interview date (yes or no answer).

Exposure (vaccination)

Target groups, vaccines in use

For both seasonal and pandemic vaccine, the target groups for vaccination were all individuals belonging to a risk group (see below). For pandemic vaccine, also the essential professionals were considered among the target group.

During the 2009-2010 influenza season seasonal vaccines were available at pharmacies and several brands were in use, namely:
Respecting to pandemic vaccines, only one vaccine was in use, Pandemrix, GlaxoSmithKline, and it was available only at vaccination centres (Health Centres from National Health Service).

**Vaccination campaign**

Seasonal vaccination campaign started on the beginning of September 2009.

The Pandemic vaccination programme in Portugal began on October 26th (week 44-45) with the group of essential professionals, pregnant women’s and individuals with chronic conditions with age between 6 months and 65 years of age. On week 47 the vaccination campaign was extended to the group of children’s with age between 6 months and 2 years and finally on week 51 to all children with more than 6 months and less than 12 years.

**Definition of vaccinated individual**

Seasonal vaccinated individuals:

- Individuals that had taken the seasonal vaccine (one of the available brands) 14 days before the disease onset;

Pandemic vaccinated individuals:

- Full vaccinated – individuals with less than 10 years or immunocompromised that took the second dose of vaccine 14 days prior the onset of symptoms and all others patients with one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms;

- Full or partial vaccinated – individuals that has received at least one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms.
**Vaccine status ascertainment**

Inoculation with 2009/2010 WHO approved influenza vaccine has been ascertained by the GPs by consulting the patient record and confirming explicitly with the patient if the vaccine was taken. If no data existed in the clinical record, patients were asked about vaccine inoculation status. Flu patients have been asked if the inoculation was through a “shot”. The day and month of inoculation have been recorded and/or asked.

**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.

For the seasonal and pandemic vaccines, risk groups were all patients with one of the following underlying conditions:

1. **Diabetes**: if treated for insulin or non-insulin-dependent diabetes;
2. **Cardiovascular disease** (myocardial infarction, angioplasty, coronary artery bypass surgery, stroke, transient ischemic attacks, treated hypercholesterolemia, treated hypertension, treated hypercholesterolemia);
3. **Chronic cardiac failure**;
4. **Chronic renal disease** (chronic renal failure and nephrotic syndrome);
5. **Chronic hepatic disease** (cirrhosis, biliar atresia and chronic hepatitis)
6. **Chronic respiratory disease** (asthma, chronic bronchitis, emphysema, brochopulmonary dysplasia, cystic fibrosis, pneumoconiosis and pulmonary fibrosis)
7. **Immunodeficiency** (conditions that suppress the immune function due to underlying disease and/or therapy, e.g. chemotherapy, HIV infection);
8. **Pregnant women in the second and third trimester.**

For the pandemic vaccine only,

1. **Morbid obesity** (≤10 years and IMC ≥ 25; >10 and < 18 years and IMC ≥ 35; adults ≥ 18 years and IMC ≥ 40)
Confounding factors and effect modifiers

Data on confounding factors and effect modifiers were collected using a standardised questionnaire. For **Cases** and **Controls** selected at GP practices, data was collected on a face-to-face interview.

The questionnaire (in annex B) was elaborated in order to collect information on the risk groups plus the following variables:

- *Previous influenza vaccination (2007-8, 2008-9)*: vaccination against seasonal influenza in the last two seasons (vaccination information for each influenza season);
- *Pneumococcal vaccination*: vaccination against pneumococcus, year of last dose of this vaccine;
- *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 status*: 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;
- *Number of GP visits in previous year*: in order to document and control for health seeking behavior the number of all GP visits in the 12 months before inclusion in the study were recorded.
- *Functional status*: low functional status was defined as needing help to bathe or to walk.
- *Antiviral administration*: use of antivirals was documented when applicable. Type and date of administration was registered.

Sample size calculation

For each VE studies (seasonal and Pandemic H1N1 2009), the sample size was planned to detect a vaccine effectiveness of 70%, considering that the vaccine coverage in the control group is 60%, with $\alpha$ error of 0.05 and a power of 90%. In these conditions at least 70 cases and controls are needed.

Considering that the proportion of ILI cases positive for seasonal influenza in the ILI cases with 65 or more years of age is expected to be 35%, according to previous results from the routine surveillance in Portugal, the total number of ILI cases required for the study is at least 200 ($=1/0.35\times70$).

For the Pandemic H1N1 2009 VE study, the sample size will be also set at 200 ILI cases from all ages. Although the proportion of ILI cases positive for A(H1N1)v in Portugal from April 2009 to September 2009 was 31% it was consider that during the 2009/2010 influenza season this proportion will be equal or higher than the observed during the previous influenza seasons.

With this design - 70 Cases versus 130 Controls group 1 – a VE 95% confidence interval of 42% to 83% is expected.
During the 2008-2009 study, where 9 GP participated with 42 ILI cases, an average of 5 ILI cases by each GP was obtained. For the current study, given that ILI cases will be included from all ages and that the Pandemic (H1N1) 2009 can configure an attack rate of 20%, approximately 3 to 4 times the attack rate of a seasonal influenza epidemic, it was considered that the number of ILI eligible cases for the study will increase. In this context it is consider that each GP can contribute with approximately 10 ILI cases in average, which is sufficient to achieve the expected sample size. In summary, it was planned to enrol 60 GP to collect at least 400 ILI cases for both VE studies to obtain 70 ILI Cases positives with 130 ILI negative cases.

Data

Data collection for cases and controls

Data on Cases and Controls were collected at GP office level. GPs interviewed the patients using a standardized questionnaire (in annex B). Each participating GP filled in the Case or Control questionnaire that included data on:

1. Demographics;
2. Signs, symptoms, date of onset of ILI;
3. Laboratory results;
4. Antiviral administration;
5. Current season influenza vaccination;
6. Previous influenza vaccination (2007-8, 2008-9);
7. Pandemic vaccination;
8. Pneumococcal vaccination;
9. Pregnancy (only for pandemic VE study);
10. Morbid obesity;
11. Smoking status;
12. Selected underlying chronic conditions;
13. Number of hospitalizations in the last 12 months;
14. Number of GP consultations in the last 12 months;
15. Number of completed years of education
16. Number of co-habitants
17. Functional status
Transmission

On a daily basis, biological material (from the swab collection) and data from ILI cases were sent by mail to the National Institute of Health where it was centrally collected. Laboratory results obtained by the Department of Infectious Diseases team were sent to the Department of Epidemiology team with ILI case code and influenza test results on a weekly basis.

In order to perform the pooled analysis of the data gathered by all the participating countries, data was also transmitted to Epiconcept. This transmission involved the data anonymization and codification according to the list of variables, definitions and coding previously provided to EpiConcept.

Entry

Final data entry was performed at Department of Epidemiology of the National Institute of Health on a SPSS database by typing in the answers from the questionnaires and laboratory results.

Validation

Before data entry, a visual verification of missing and inconsistent values was done by the research team; after data entry a validation script was also run on the database. Validation procedures included verification of the presence of impossible values and of possible inconsistencies in variables and between variables. All missing or inconsistent values where clarified with the corresponding GP.

Finally double data entry was performed at the end of study. Values found incongruent were checked in paper questionnaires and corrected in the final database.

Data cleaning

All ILI cases that did not meet the EU ILI criteria were excluded from analysis. Comparison between the values from the paper questionnaires and the data entered on the database was performed. When inconsistencies were found, the corresponding GP was contacted in order to clarify the data.
Analysis

Coding and categorization of variables

All categorical variables were previously coded with exception to:

- the age group was created from the variable age and categorized in four classes: 0-4; 5-14; 15-64 and ≥65 years of age;
- the indicator variable of the delay between the onset of disease and swab less than 3 days data was computed from the number of days between the onset and the swab;
- the smoking status variable was recoded as 1- current smoker and 0- former and never smoker;

The variables treated as numerical (discrete or continuous) were age, days between the onset of the symptoms and swab, number of previous hospitalizations due to the underlying chronic diseases in the last 12 months, number of education years, number of co-habitants and number of GP consultations in the last 12 months.

Exposure to seasonal influenza vaccine variable:

Vaccinated (coded 1)- ILI case has taken the seasonal vaccine 14 days before the disease onset; Not vaccinated (coded 0)-all others

Exposure to pandemic influenza vaccine (two variables)

1) Full exposure variable

Vaccinated (coded 1) - ILI case with less then 10 years or immunocompromised that has taken second dose of vaccine 14 days prior the onset of symptoms and all others ILI cases with one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms; Not vaccinated (coded 0)-all others.

2) Full or partial exposure variable

Vaccinated (coded 1) - ILI case that has received at least one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms; Not vaccinated (coded 0)-all others.

Comparison of group’s characteristics

Cases (ILI positive) and Controls (ILI negative) were compared according to the following variables: age, sex, pregnancy, morbid obesity, smoking status, diabetes, cardiovascular disease, heart failure, renal failure, chronic hepatic disease, immunodeficiency, previous seasonal vaccines (2008-2009, 2007-2008), help for bathing, number of hospitalizations in the previous 12 months, years of education, patient belong to the GP list, number of GP consultation in the previous 12 months, number of co-habitants and
the ILI symptoms.

The comparisons were performed considering that the samples were independent.

Association between variable Case/Control and the categorical variables was evaluated by the Chi-squared test. If at least one of the table cells presented expected frequencies lower than 5, the Chi-squared test was substitute by the Fisher’s Exact test.

Comparisons of numerical variables between groups (Case/Control) were performed using the non parametric test of Mann-Whitney.

**Measure of effect**

The vaccine effectiveness was computed as VE=1-OR (crude) and aVE=1-aOR (adjusted) where OR and aOR is respectively the crude and adjusted odds ratio of being vaccinated within Cases versus Controls.

For the crude estimate, the exact 95% confidence interval of VE (OR) was obtained by the method described in Sahai H and Khurshid\(^\text{12}\). The confidence interval for the aVE was computed by the respective method of adjustment (non conditional Logistic Regression).

Vaccine effectiveness (crude and adjusted by age group) was also computed by comparing the proportion of vaccinated Cases with the vaccine coverage estimated on the Community control group using the screening method as described Farrington 1993\(^\text{13}\).

**Stratified analysis**

Due to the low number of cases, a stratified analysis was not performed.

**Multivariable analysis**

The odds ratio of being vaccinated within Cases versus Controls was adjusted for possible confounders. Adjustment was performed by using the non conditional logistic regression.

**Restricted analysis**

Restricted analysis was performed for the ILI cases swabbed within 3 days of the disease onset and for ILI cases with less than 65 years of age.

**Software used for data entry, statistical analysis.**

All the results were obtained using the package of statistical programs PASW Statistics 18.0\(^\text{14}\).
Logistical aspects

Consent

Each GP had the responsibility of asking oral consent from ILI cases and controls, after giving adequate information on the general study characteristics.

Ethical approval

The study protocol was submitted and approved by the Comissão de Ética (Ethics Committee) of Instituto Nacional de Saúde Dr Ricardo Jorge, I.P (annex A).

Team

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General Directorate of Health

Isabel Falcão

MS network

Supervision

A supervising committee was established with participating members of the Direcção-Geral da Saúde (General Directorate of Health), INFARMED (National Authority of Medicines and Health Products), CEFAR/ANF (National Pharmacies Association) and APMCG (Portuguese Association of General Practitioners).

Training

After the selection procedure, to each one of the GPs that agreed to participate, a personal interview was made by phone explaining the study and their participation. They also received the protocol, case definition questionnaires and laboratory swabbing procedures.

To all, has been described:

- the design of the project;
- the ECDC case definition;
the inclusion and exclusion criteria to select ILI cases and underlined that selection should be independent of vaccination status;

• the definitions and concepts associated to each variable in the questionnaires and the way of answer or coding questions;

• to collect nasopharyngeal samples, and provide transportation to the National Influenza Reference laboratory in INSA;

• to accept data quality checks on the quality of some selected issues.

For these purposes several telephone calls have been made during the recruitment and development of the study. When necessary, some personal contacts or by e-mail have been made to clarify doubts.
Results

Participating GP’s:

After the selection procedure, 53 GPs agreed to participate. About 60% (32) participated in the study by selecting, collecting swabs and data on Cases and Controls.

Results from a telephone and email survey conducted to the 21 GP’s that did not send any ILI case showed that the main reason was the fact that no ILI case was detected among their medical consultations, representing 86% of the respondents GPs, response rate approximately 33%.

As mentioned, the total number of GPs participating in the study was 53, 23 were current active members of the MS network, whilst 30 GPs were ex-members of the network or others (Table 1).

Table 1- Number of GPs that accepted to participate and those that provided at least one pair ILI case / control.

<table>
<thead>
<tr>
<th></th>
<th>GPs currently participating in MS¹</th>
<th>GPs ex-participants in MS or others¹</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>All GPs accepting to participate</td>
<td>23</td>
<td>30</td>
<td>53</td>
</tr>
<tr>
<td>GPs reporting valid data</td>
<td>15</td>
<td>17</td>
<td>32</td>
</tr>
</tbody>
</table>

¹. MS – “Médicos-Sentinela” network

All participating GPs work in a Health Center of National Health Service (Ministry of Health) and have a stable list of patients. GPs that accepted to participate in Euroeva were distributed by all 5 Administrative Regions and by 15 of the 18 Districts of mainland Portugal. GPs reporting ILI cases covered all 5 regions and 11 of the Districts (Figure 1).
Figure 1: Distribution of participating a) and effectively reporting b) GPs.

Regarding the population covered by the Euroeva project, obtained by the sum of all the patients belonging to GP lists, it can be seen in Table 2 that no major difference was found between the Euroeva age group distribution and the one observed in all MS network and the 2009 Portuguese population estimates. In detail, between Euroeva study population and population estimates, only a small difference (approximately 2%) is found in the extreme age groups, with 0-14 years age group underrepresented and the 65+ age group overrepresented.

Table 2 – Distribution of the population covered by the Euroeva project and MS network compared with 2009 Portuguese population estimates.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>EUROEVA</th>
<th>MS network</th>
<th>Population 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>13.5%</td>
<td>15.3%</td>
<td>15.2%</td>
</tr>
<tr>
<td>15-24</td>
<td>11.3%</td>
<td>11.5%</td>
<td>11.1%</td>
</tr>
<tr>
<td>25-64</td>
<td>54.8%</td>
<td>54.8%</td>
<td>55.8%</td>
</tr>
<tr>
<td>65+</td>
<td>20.5%</td>
<td>18.5%</td>
<td>17.9%</td>
</tr>
</tbody>
</table>
Influenza season 2009-2010

Virus circulation

The influenza activity in Portugal, during the 2009-2010 influenza season, was associated with the predominant circulation of the pandemic influenza virus A(H1N1)v. The pandemic virus was detected from April, 2009 (week 17) through mid February, 2010.

Duration

By week 41 of 2009 the flu incidence rate began to rise quickly, peaked at week 46 with 136.4/10^5 inhabitants, and after decreased progressively till week 2 and stayed below the baseline until the end of the season. The incidence rate stayed above the baseline threshold for 7 weeks from week 44 to week 51.

ILI incidence, severity

Comparing the epidemic period of 2009-2010 with the previous 2008-2009, they are similar in duration and the incidence rates in the peaks are similar, respectively 136.4 and 142.3/10^5 inhabitants (Figure 2). The main difference respects to 1) the dominant subtype of influenza virus in 2008-2009 which has been a seasonal influenza A and just a few Influenza B while the 2009-2010 season has been characterized by a complete dominance of the A(H1N1)2009 and the higher incidence rates in the age groups below 14 years in the season 2009-2010 (Figure 3).

Figure 2: Distribution of provisional incidence rates and number of virus detected by week in 2008-2009 and 2009-2010 seasons
Figure 3: Distribution of provisional incidence rates by age group during 2008-2009 and 2009-2010 seasons.

Vaccination

Seasonal vaccination campaign started on the beginning of September 2009.

The 2009-2010 seasonal influenza vaccine coverage estimates for individuals aged 65 years or more was 52.2% (IC95% [46.4-59.6]), according to the yearly conducted dual-frame telephone survey\textsuperscript{10}.

Pandemic vaccination programme in Portugal began on 26th October (week 44-45) with the group of essential professionals, pregnant women and individuals with chronic conditions with age between 6 months and 65 years of age. On week 47 the vaccination campaign was extended to the group of children's with age between 6 months and 2 years and finally on week 51 to all children with more than 6 months and less than 12 years.

The evolution of the pandemic vaccine coverage in the general mainland Portugal population is presented in Figure 4. The vaccine coverage was 1.4% at the start of the pandemic VE study (week 50) and has reached 5.5% in week 12.
Figure 4: Evolution of the pandemic influenza vaccine coverage in the general population. Portugal Mainland (Information source Direcção-Geral da Saúde).
Description of participants

For the seasonal VE study, 63 ILI cases were enrolled, from these 10 were excluded (4 did not meet the age criteria and 6 the EU ILI case definition).

For the pandemic VE study 244 ILI cases were enrolled, from these 56 were excluded given that did not meet ILI EU criteria, were included prior the beginning of the pandemic vaccine campaign, days between symptoms and swabbing, onset week where the last case was detected criterion. Given this, the final analysis included 188 ILI cases from all ages.

Laboratory results

In the context of this study 244 specimens were collected, distributed in time as shown in Figure 5, where 6 cases are not plotted due to missing date of symptoms onset. The occurrence of 34 confirmed cases for pandemic Influenza A (H1N1) and 210 influenza negative cases is shown.

![Figure 5: Weekly distribution of the 238 cases analyzed](image)

Specimens were collected from week 46/2009 to 14/2010 and all confirmed cases were associated with the influenza A(H1N1) pandemic virus (Figure 6). No seasonal influenza A(H1N1), A (H3N2) and B viruses were detected.
Figure 6: Characterization of ILI cases. The positive cases account for 13.9% of the total cases analyzed, all associated with the pandemic Influenza A (H1N1).

Pandemic Influenza A(H1N1) virus isolates were obtained from 16 specimens. These were all antigenic and genetically closely related to the vaccine virus A/California/7/2009 (figure 6, 7).

Figure 7: Phylogenetic relationship of influenza A(H1N1) pandemic viruses HA1 subunit. The tree was constructed by Clustal Method with the Megalign program (DNASTar 99 package, Lasergene). All pandemic A(H1N1)2009 influenza viruses analysed in this study (in blue) are genetically closely related to the vaccine virus A/California/7/2009 (in red) (Influenza Virus Resource, NCBI).
Figure 8: Phylogenetic relationship of influenza A(H1N1) pandemic viruses neuraminidase gene. The tree was constructed by Clustal Method with the Megalign program (DNASTar 99 package, Lasergene). All pandemic A(H1N1)2009 influenza viruses analysed in this study (in blue) are genetically closely related to the vaccine virus A/California/7/2009 (in red) (Influenza Virus Resource, NCBI).

Ten isolates were studied, all having the amino acid changes S203T, I321V and P83S on the HA1 subunit of the haemagglutinin. Five isolates had the D222E substitution, but none possess the D222G amino acid change. In spite of these amino acid changes, all isolates remained antigenically indistinguishable from the A/California/7/2009 vaccine virus.

Phenotypic assays to evaluate the antiviral resistance, conducted at the WHO collaborating Centre at Mill Hill, London, confirmed that the isolates studied were sensible to oseltamivir. These results were expected since the genetic analysis conducted in the National Reference Laboratory didn’t detected the aminoacid substitution H275Y on the neuraminidase gene.

Description of Cases and Controls

For analysis purpose data will be restricted to the 188 ILI cases obtained after exclusion criteria application. From the 188 ILI cases enrolled in the study 32 that were positive for influenza A(H1N1)v pandemic virus, will be considered as Cases and 156 that test-negative as Controls.

On average Cases (mean 36yrs) were younger than Controls (mean 46yrs) and this difference was found to be statisticaly significant. Also to notice that 12.5% of Cases and 31.4% for the Controls group were 65 or more years of age.
Significant differences were also found on:

- the prevalence of cardiovascular diseases, with Controls (29.0%) presenting a higher proportion of patients with these conditions than Cases (6.3%);
- the prevalence of patients with chronic renal disease was higher in Cases (6.3%) than in Controls (0.0%);
- the proportion of patients that belonged to the attended GP patient list was higher in Controls (52.6%) than in Cases (31.3%);
- the median number of general practitioner consultations in previous 12 months, higher in Controls (4) than in Cases (3) and
- the median number of co-habitants also higher in Cases (3) than in Controls (2).

The proportion of Cases with morbid obesity was also higher in Cases (9.4%) than in Controls (1.9%) although with a borderline statistical significant difference.

When comparing symptoms and signs between Cases with Controls, only fever (above 38°C) has showed a significant difference: here 96.8% of Cases and 78.7% Controls presented this symptom.

No differences were found in the time between the onset and the swab between Cases and Controls and none of the ILI cases enrolled in the study had been previously treated with antiviral drugs.
**Table 3:** Description of cases and control, week’s 46-7 influenza season 2009-2010, by age, sex, pregnancy, morbid obesity, smokers, pneumococcal vaccine, seasonal vaccine in previous years, chronic conditions, GP consultations and hospitalizations in the last 12 months, years of education and number of co-habitants.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean</td>
<td>36 (32)</td>
<td>46 (156)</td>
<td>0.017$^1$</td>
</tr>
<tr>
<td>0-4, %</td>
<td>0.0</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>5-14, %</td>
<td>15.6</td>
<td>4.5</td>
<td>0.007$^3$</td>
</tr>
<tr>
<td>15-64, %</td>
<td>71.9</td>
<td>56.4</td>
<td></td>
</tr>
<tr>
<td>≥65, %</td>
<td>12.5</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>Sex, male %</td>
<td>37.2 (32)</td>
<td>43.8(156)</td>
<td>0.486$^3$</td>
</tr>
<tr>
<td>Pregnant women's, %</td>
<td>0.0 (13)</td>
<td>6.5 (46)</td>
<td>1.000$^2$</td>
</tr>
<tr>
<td>Morbid obesity, %</td>
<td>9.4 (32)</td>
<td>1.9 (156)</td>
<td>0.059$^2$</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>12.5 (24)</td>
<td>17.6 (131)</td>
<td>0.768$^2$</td>
</tr>
<tr>
<td>Pneumococcal vaccine, %</td>
<td>0.0 (29)</td>
<td>7.3 (150)</td>
<td>0.216$^2$</td>
</tr>
<tr>
<td>Seasonal flu vaccine 2008-09</td>
<td>24.1 (29)</td>
<td>29.3 (140)</td>
<td>0.576$^3$</td>
</tr>
<tr>
<td>Seasonal flu vaccine 2007-08</td>
<td>23.1 (26)</td>
<td>26.5 (136)</td>
<td>0.718$^3$</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>6.5 (31)</td>
<td>10.3 (155)</td>
<td>0.742$^2$</td>
</tr>
<tr>
<td><strong>Cardiovascular diseases, %</strong></td>
<td>6.3 (32)</td>
<td>29.0 (155)</td>
<td>0.007$^3$</td>
</tr>
<tr>
<td>Heart failure, %</td>
<td>0.0 (32)</td>
<td>2.6 (155)</td>
<td>1.000$^2$</td>
</tr>
<tr>
<td><strong>Chronic renal disease, %</strong></td>
<td>6.3 (32)</td>
<td>0.0 (155)</td>
<td>0.029$^2$</td>
</tr>
<tr>
<td>Chronic hepatic disease, %</td>
<td>0.0 (32)</td>
<td>0.6 (154)</td>
<td>1.000$^2$</td>
</tr>
<tr>
<td>Chronic respiratory disease, %</td>
<td>6.3 (32)</td>
<td>14.2 (155)</td>
<td>0.382$^2$</td>
</tr>
<tr>
<td>Help for bathing, %</td>
<td>0.0 (32)</td>
<td>10.3 (156)</td>
<td>0.078$^2$</td>
</tr>
<tr>
<td>Belongs GP patient list, %</td>
<td>31.3 (32)</td>
<td>52.6 (156)</td>
<td>0.028$^3$</td>
</tr>
<tr>
<td>GP consultations last 12 mo, median</td>
<td>3 (29)</td>
<td>4 (148)</td>
<td>0.024$^1$</td>
</tr>
<tr>
<td>Hospitalizations, median</td>
<td>0 (28)</td>
<td>0 (150)</td>
<td>0.533$^1$</td>
</tr>
<tr>
<td>Years of education, median</td>
<td>6 (30)</td>
<td>4 (1)</td>
<td>0.364$^1$</td>
</tr>
<tr>
<td>Co-habitants, median</td>
<td>3 (32)</td>
<td>2 (154)</td>
<td>0.016$^1$</td>
</tr>
</tbody>
</table>

(1), number of valid answers; 1, Mann-Whitney test; 2, Fisher’s Exact test; 3, Chi-squared test
Table 4: Description of cases and control, week’s 46-7 influenza season 2009-2010, by time between the onset and swab, the symptoms, signs and treatment with antiviral.

<table>
<thead>
<tr>
<th>Time between onset and swab collection (days),</th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>2.1</td>
<td>2.2</td>
<td>0.584¹</td>
</tr>
<tr>
<td>less than 3 days, %</td>
<td>84.4 (32)</td>
<td>82.1 (156)</td>
<td>0.753³</td>
</tr>
<tr>
<td>Fever, %</td>
<td>96.8 (31)</td>
<td>78.7 (141)</td>
<td>0.018³</td>
</tr>
<tr>
<td>Malaise, %</td>
<td>81.3 (32)</td>
<td>81.9 (155)</td>
<td>0.927³</td>
</tr>
<tr>
<td>Headache, %</td>
<td>80.6 (31)</td>
<td>80.3 (147)</td>
<td>0.962³</td>
</tr>
<tr>
<td>Myalgias, %</td>
<td>81.3 (32)</td>
<td>80.4 (143)</td>
<td>0.963³</td>
</tr>
<tr>
<td>Cough, %</td>
<td>90.6 (32)</td>
<td>82.7 (156)</td>
<td>0.264³</td>
</tr>
<tr>
<td>Sore throat, %</td>
<td>77.4 (31)</td>
<td>85.7 (154)</td>
<td>0.279²</td>
</tr>
<tr>
<td>Shortness of breath, %</td>
<td>13.3 (30)</td>
<td>21.1 (147)</td>
<td>0.331³</td>
</tr>
<tr>
<td>Treatment with antiviral, %</td>
<td>0.0 (27)</td>
<td>0.0 (145)</td>
<td>-</td>
</tr>
</tbody>
</table>

¹, number of valid answers; 1, Mann-Whitney test; 2, Fisher’s Exact test; 3, Chi-squared test

Vaccine coverage

None of the Cases was vaccinated for pandemic vaccine; on the other hand, the pandemic vaccine coverage on Controls was 4.5% for full vaccination criteria and 6.4% for full or partial vaccinated (only one dose taken for those that two doses were recommended). The seasonal vaccine coverage was higher in Controls (33.3%) than in Cases (25.0%).

Table 5: Description of cases and control (test-negative design), week’s 46-7 influenza season 2009-2010, by vaccine coverage.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pandemic vaccine coverage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full vaccinated, %</td>
<td>0.0 (32)</td>
<td>4.5 (156)</td>
<td>0.605¹</td>
</tr>
<tr>
<td>Full or partial vaccinated, %</td>
<td>0.0 (32)</td>
<td>6.4 (156)</td>
<td>0.216¹</td>
</tr>
<tr>
<td>Seasonal vaccine coverage</td>
<td>25.0(32)</td>
<td>33.3 (156)</td>
<td>0.357²</td>
</tr>
</tbody>
</table>

¹, Fisher’s Exact test; 2, Chi-squared test

Full vaccinated: patients with less than 10 yrs or immunocompromised are considered vaccinated if the second dose of vaccine was taken 14 days prior the onset of symptoms all others one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms.

31
**Table 6:** Description of cases and community control (screening method), week’s 46-7 influenza season 2009-2010, by vaccine coverage.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Community controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pandemic vaccine coverage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;=18 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full or partial vaccinated, %</td>
<td>0.0 (24)</td>
<td>4.3 (969)</td>
</tr>
<tr>
<td><strong>Seasonal vaccine coverage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(all ages)</td>
<td>25.0 (32)</td>
<td>16.7 (2809)</td>
</tr>
</tbody>
</table>

*Full or partial vaccinated: the patient has received at least one dose of the pandemic vaccine, at least 14 days prior the onset of symptoms.

* estimates not adjusted for population distribution.

**Vaccine effectiveness**

**Pandemic vaccine effectiveness**

Given that all the Cases were not vaccinated with the pandemic vaccine this estimate is not possible to obtain. No statistical association was found between pandemic vaccine and medical attended A(H1N1)v influenza infection.

**Seasonal vaccine effectiveness against A(H1N1)v infection**

**Test-negative design**

Crude vaccine effectiveness varied from 5% to 43%, for the different restrictions criteria. All these estimates presented very wide 95% confidence intervals. After the adjustment for confounders, via non conditional logistic regression, the VE estimates varied from 29% to 38%. None of these estimates was statistical significant, all of them presenting a very low precision. Nevertheless it’s important to state that all the point estimates pointed for a low protective effect of seasonal vaccine on medical attended influenza A(H1N1)v infection.
Table 7: Crude and adjusted seasonal vaccine effectiveness against infection by A(H1N1)v estimates based on comparison of laboratory-confirmed influenza case subjects and test-negative control subjects, influenza season 2009-10, Country Portugal.

<table>
<thead>
<tr>
<th>Cases/Controls</th>
<th>OR</th>
<th>VE</th>
<th>IC95% (%)</th>
<th>Cases/Controls</th>
<th>OR</th>
<th>VE</th>
<th>IC95% (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No restrictions(^1)</td>
<td>32/156</td>
<td>0.67</td>
<td>33%</td>
<td>-59; 72</td>
<td>23/132</td>
<td>0.71</td>
<td>29%</td>
</tr>
<tr>
<td>Less than 4 days onset to swab(^1)</td>
<td>27/128</td>
<td>0.57</td>
<td>43%</td>
<td>-50; 79</td>
<td>18/107</td>
<td>0.68</td>
<td>32%</td>
</tr>
<tr>
<td>Age group 0 – 64 yrs(^2)</td>
<td>28/107</td>
<td>0.95</td>
<td>5%</td>
<td>-179; 68</td>
<td>20/87</td>
<td>0.62</td>
<td>38%</td>
</tr>
</tbody>
</table>

OR adjusted for 1. age group, previous seasonal vaccination 08 or 07, chronic diseases, GP visits in the last 12 months, nbr of co-habitants, patient belonged to the GP list, month of disease onset;

Screening method

The estimated effect of the 2009-2010 seasonal vaccine using the screening method was not statistically significant: -66% (IC95% -270; 25) unadjusted and -87% (IC95% -350; 21) adjusting for age. These results reflect the fact that the seasonal vaccine coverage in the general population was lower than the observed in the Cases group.
Discussion

Overall results

Seasonal vaccine effectiveness

The total number of ILI cases eligible for the seasonal VE study (aged 65 yrs or more) was 53, none of these were positive for any of the seasonal influenza virus, i.e., the study was unable to find Cases for the seasonal VE study. This result precluded the possibility of estimating seasonal vaccine effectiveness.

No seasonal influenza viruses were detected in Portugal in the context of the National Influenza Surveillance Programme. However, when considering National data from other sources, seasonal influenza viruses were observed in circulation in Portugal at a very low level, a fact that is consistent with reports from other European Countries.

Pandemic vaccine effectiveness

For the pandemic VE study 188 ILI patients were considered for statistical analysis, 32 were positive for the pandemic strain A(H1N1)v (Cases) and 153 were negative for this strain (Controls), which represents 17% of positives.

All pandemic A(H1N1)2009 influenza viruses analysed in this study were antigenic and genetically closely related to the vaccine virus A/California/7/2009. The genetic analysis of the ten isolated strains revealed that all have the amino acid changes S203T, I321V and P83S on the HA1 subunit of the haemagglutinin. Five isolates had the D222E substitution, but none possess the D222G amino acid change. In spite of these, amino acid changes the influenza strains remained antigenically indistinguishable from the A/California/7/2009 vaccine virus. All strains studied lack the H275Y mutation in the neuraminidase gene and therefore assumed to be sensitive to the influenza virus neuraminidase inhibitors. This assumption was confirmed by phenotypic assays performed at the WHO Collaborating Centre for Europe.

The pandemic vaccine coverage was not statistically different between Cases and Controls, considering any of the vaccination definitions (Full or Full and partial). Specifically, the coverage of the pandemic vaccine was 0% on Cases, meaning that none of the Cases was vaccinated with pandemic vaccine.

Considering the above mentioned results, it was not feasible to calculate the pandemic VE estimate.

This result can be explained given the fact that, in Portugal, the pandemic vaccine campaign initiated at 26 of October 2009 - week 44, in the beginning of the epidemic period. On the other hand when the ILI
incidence rate peaked at week 46 (Figure 8), two weeks after the beginning of the vaccine campaign, only 11.656 pandemic vaccines had been distributed, which represents a vaccine coverage of 0.1%. Given that, and the fact that the present study was design considering high vaccine coverage in the population, the launch of the pandemic VE study was postponed for week 1 of 2010. Meanwhile, from week 46 to week 2 the ILI incidence rate and the proportion of ILI positives for influenza virus started to decrease. This scenario forced the anticipation of the beginning of the study to week 50, given the risk of study starts after the epidemic period.

**Figure 9** – Weekly distribution of the ILI incidence rate (Sentinel system - Médicos-Sentinela network) and of the pandemic vaccine coverage (Direcção-Geral da Saúde)

In synthesis the main factors that contributed to the low vaccine coverage in Controls and the absence of vaccine failures were:

1. the early beginning of the study in the perspective of the exposure, once there was a low vaccine coverage (1.4% at the beginning and 5.5% at week 7), due to the late beginning of the vaccine campaign, and,

2. the late beginning of the study in the context of the epidemic period, given that the study started in last week of the epidemic period (week 50). In week 51 the ILI incidence rate was already below the
threshold of the baseline, with a decreased proportion of ILI positives for influenza

Given this national scenario, the pooled analysis of the I-Move case-control studies came out has the main solution in order to obtain pandemic VE estimates. The Portuguese data was, has planned, sent to the I-Move project coordination in January and April empowering the pooled analysis results.

**Effect of the seasonal vaccine on the A(H1N1)v infection**

Even though the effect of the seasonal vaccine on the A(H1N1)v virus infection was not part of the Euroeva scientific protocol, it was considered important to evaluate it, mainly because results in Canada showed increased risk of A(H1N1)v infection on individuals vaccinated with 2008-2009 seasonal vaccine\(^{15}\).

The coverage of the 2009-2010 seasonal vaccine was lower in Cases (25.0%) than Controls (33.3%), difference that was not significant.

No statistical association was found between the seasonal vaccine and the A(H1N1)v infection for all the enrolled ILI patients and also considering two different levels of data restriction (less than 4 days between onset and swab and ILI patients less than 65 years of age). The crude estimates of seasonal VE against the pandemic virus varied from 5% to 43% and the adjustment for confounders estimate varied from 29% to 38%, all of these point estimates presented very wide 95% confidence intervals.

Very wide confidence intervals were also obtained using the screening method although the point estimate was presented has a risk factor. Nevertheless it’s important to notice that in this method the control group is the general population and that it was only possible to adjust for age.

**Participation rate**

During the 2009-2010 study, 53 GP’s agreed to participate in Euroeva, from these 32 GP’s contributed with ILI cases, which represents a 60% participation rate. Results from a telephone and email survey conducted on the 21 GP’s that did not send any ILI case showed that the main reason was the absence of ILI cases in the consultation, although the survey presented a low response rate (33%).

Comparing the 2009-2010 study with the 2008-2009 pilot study, it can be seen that the GP participation rate increased from 20 to 60% and sample size from 42 ILI cases\(^6\) to 244 ILI cases. Even though, these results must be contextualized in the light of the differences between the studies (2008-2009 was specifically for elderly ILI patients) and the alert given to the Pandemic (H1N1)2009.

Comparing these results with goals stated in the protocol, the number of GP’s enrolled was lower than the 60 GP’s planned but close. The pandemic VE study sample size (244) was higher than 200 ILI cases
planned in the protocol; on the other hand the seasonal VE study sample size (53) study was lower than the 200 planned. This result show has in the 2008-2009 pilot study, the difficulty of collecting ILI patients with 65 or more years of age.

**Representativeness of GP’s and covered population**

Participating GPs were volunteers for Euroeva as they are for participating on the MS network. Therefore they do not represent the total group of GPs working in health centers, in Mainland Portugal.

Nevertheless the age distribution of population covered by Euroeva project was very similar with the age distribution observed in the MS network population and with the national estimates for 2009 (Table 2).

**ILI Cases**

During the 2009-2010 study, the adopted ILI case criteria was the EU ILI case definition increasing in this way the consistency with the remaining I-Move study sites. The implementation of the EU ILI definition was well succeed once from the 244 ILI cases enrolled only 19 did not fulfill the EU ILI criteria, meaning that 92.2% of the ILI cases were in accordance with EU ILI criteria.

Another important improvement towards a better representativeness of ILI cases was the introduction of the systematic selection of ILI patients - four by week/GP (two ILI cases aged 65+ and two cases with less than 65 years), with a different first day of the week from GP to GP that was randomly assigned. In order to help GP’s following these rules; weekly reminders were sent to GP’s on the day before the beginning of his first week day via SMS (Short Message Service). This rule was fulfilled given that the ILI cases received by GP/week ranged from 0 to 4.

ILI patients not belonging to the GP list were included in the study given that a contact GP patient had occurred. This option was also different from the 2008-2009 pilot study and adopted with objective of increasing the sample size. In true, from the 244 ILI cases enrolled 129 (53%) did not belong to the list of the GP consulted, indicating that the inclusion of these cases has doubled the expected sample size.

A GP can perform a consultation with a patient out of his list mainly due to three reasons: 1. the patient does not have an assigned GP; 2. the assigned GP of that patient was at time of consultation out of office; 3. the GP collected the ILI case at a “Serviço de Atendimento à Gripe” (“flu clinic”) that were implemented the Ministry of Health during the pandemic (H1N1)2009 in Portugal.

At this point the question about the differences between these two groups of patients and the possible impact on VE estimates must be discussed. Comparing the groups it was found that: 1. the ILI patients that belonged to the GP list were younger (median 38 vs 53, p=0.048), presented a lower seasonal and
pandemic vaccine coverage (29% vs 36% and 4% vs 10%, not statistical significant) and a higher proportion of laboratory confirmed cases (23% vs 11%, p=0.028).

These results underline in one hand the increase on the representativeness ILI patients that seek medical care in the study, once the included group was not represented in the study if only ILI cases from the GP list were enrolled, and of course the importance of stratify or adjust the VE results for this variable.

**Controls**

In the 2009-2010 study only two groups of controls were considered in order to estimate VE. The ILI influenza negative cases and the community sample obtained by an independent routine procedure used to estimate seasonal vaccine coverage in population\(^1\).

The ILI influenza negative controls were particularly interesting since they were obtained directly from the routine surveillance system, just adding a number of variables to be used mainly in stratified analysis, effect modification and confounding. Also of substantial interest is the community control group selected from the Portuguese general population directly from an independent routine source that is easily accessed since is a national routine system to estimate seasonal vaccine coverage.

It would be very important that the percentage of vaccinated reached an equivalent level between the two control groups. The pandemic vaccine coverage in the ILI negative control group was 6.4 (IC95%:2.6; 10.3%) and if restricted to the population 18 years or more, 4.5% (IC95%: 1.0; 8.0%). This last figure is consistent with the estimated pandemic vaccine coverage in the community control group obtained for the same age group, 4.3% (IC95%: 3.1; 5.6%). All these estimates were in accordance with the vaccine coverage obtained from the national registries (Direcção-Geral da Saúde) that points to a vaccine coverage of 5.5%.

Regarding the seasonal vaccine coverage, the consistency between the two control groups was not verified (ILI negative group: 33.3% [IC95%: 26.0; 41.0%] and community group: 16.7% [IC95%: 15.2; 18.0]). One possible explanation for this difference may be the significant difference between the age distributions observed in the two control groups.
Conclusions

The current study was unable to provide pandemic and seasonal influenza vaccine effectiveness estimates at the national level. This was mainly due to the low number of influenza cases enrolled and that none was vaccinated for the pandemic strain.

This situation has also occurred in other I-MOVE site studies; nevertheless the joint effort of the multicentre sites has enabled the estimation of a pandemic VE with a reasonable precision.

Another objective that was added to the study protocol was the effect of 2009-2010 seasonal vaccine on the A(H1N1)v infection. The results of this study were unable to find any association between 2009-2010 seasonal vaccine with the A(H1N1)v infection.

Regarding study design and logistic aspects, the 2009-2010 Euroeva study has achieved the following results:

1) the introduction of the EU ILI case definition has the ILI case selection criteria, was well succeed since 92% of ILI cases respected the case definition;

2) the introduction of the systematic selection of ILI patients - four by week, with the first day of the week different from GP to GP;

3) the absence of substantial departures from the original scientific protocol;

4) the introduction of weekly reminders sent to GP’s on the day before the beginning of his week via SMS (Short Message Service) has allowed a close follow-up ILI cases collection and helped the prosecution of the systematic selection by the GP’s;

5) participation rate and sample size: the increase of the GP participation rate from 20% in 2008/2009 to 60% and sample size from 42 ILI cases to 244 ILI cases. Even though, these results must be contextualized in the light of the differences between the studies (2008-2009 was specifically for elderly ILI patients) and the alert given to the Pandemic (H1N1)2009.

6) the inclusion of the ILI cases out of the GP patient list has enhanced the representativeness of ILI cases from the general population.
The main recommendations focused on:

- To increase sample size, mainly in the elderly population (aged 65 years or more);
- To increase the total number of participating GP's in the study by exploring other sources of GP's recruitment;
- To register if the enrolled ILI cases belongs to the influenza vaccine recommended target groups at the national level;
- To start the study as soon as possible taking into consideration the beginning and evolution of the vaccination campaign;

Finally we also recommend continuing the harmonization of the study designs between participating countries with the multi-centre study objective.
Aknowledgments

The authors would like to acknowledge all the GPs participating in this study:

Aldora Firmo, Ana Cristina Pinto Magalhães, António Ferreira da Cunha, António Gabriel Ferreira Santos, António João Passão Lopes, António José da Silva Valente, António Mendes da Luz, Cecília Teixeira, Cláudio Carril, Dorinda Maria Carvalho Calha, Elisa Maria Bento da Guia, Emília Barros, Fátima Valente, Fernanda Maria Gomes Ferreira, Fernando Ferreira, Fernando Manuel Oliveira Fardilha, Francisco Araújo, Hermínia Jesus Soares Nascimento, Inês Marcos, Isabel Alves, Isabel Taveira Pinto, Jaime Correia de Sousa, João Adélio Trocado Moreira, João Ricardo Santos Brito, José Augusto Rodrigues Simões, José Manuel Gonçalves Silva, José Manuel Oliveira Santos, Lia Cardoso, Luís Miguel Monteiro, Luísa Fernandes, Madalena de Sá Ribeiro Cubal, Manuel dos Santos Soares, Margarida Brito, Maria Antónia Dias Cruz, Maria da Conceição Fraga Costa, Maria da Luz Esteves, Maria Elvira Pinto Costa Silva, Maria Isabel Carvalho Azeredo Lobo, Maria Manuel Marques Açafrão, Maria Manuela Moreira Sucena Mira, Maria Teresa Simões Brandão, Maria Teresa Vieira Libório, Marília José Pereira Diogo, Mário Silva, Olga Xavier, Paulo Ascensão, Paulo Goucha, Rosa Gallego, Rui Nogueira, Sara Marques, Sérgio Vieira, Sónia Cruz e Vítor Manuel Jesus Vaz Moreira.

Also, the authors would like to acknowledge Associação Portuguesa de Médicos de Clínica Geral.
References

Annexes

Annex A - Project submission to the Ethics committee of the Instituto Nacional de Saúde Dr. Ricardo Jorge
De: Secretariado da Comissão de Ética

Para: Baltazar Nunes

Assunto: Pedido de apreciação e parecer – projecto Euroeva

No seguimento do seu pedido de apreciação e parecer, relativo ao projecto de investigação Euroeva, vimos por este meio informar que o mesmo mereceu parecer positivo da Comissão de Ética deste Instituto em reunião realizada no passado dia 23 de Abril, aproveitando para desejar o maior sucesso no desenvolvimento deste trabalho.

Com os melhores cumprimentos,

O Secretariado da Comissão de Ética

[Stamp: Comissão de Ética INSA, I.P.]
Annex B – Questionnaires
Código do caso: 0101_001_2

Efectividade da Vacina Antigripal EuroEva - 2009/2010

Notificação Clínica da Síndroma Grippal
Colheita de Produto Biológico

Informação Relativa ao Doente
Nome: ..........................................................................................................................
Sexo: ..........................................................................................................................
Idade: ..........................................................................................................................
Nº Cartão de utente/ Proc. Clínico: ..............................................................................
Telefone: ....................................................................................................................

Síndroma Grippal
Nova definição de caso, ECDC: A +1 sint. de B +1 sint. de C

A
Data de início dos sintomas: ......................................................................................

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<thead>
<tr>
<th>Início súbito (&lt;24h)</th>
<th>[SIND]</th>
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B

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<th>Febre ou febrícula</th>
<th>___ ºC</th>
<th>[SIND]</th>
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<thead>
<tr>
<th>Mal-estar geral, debilidade, prostração</th>
<th>[SIND]</th>
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<th>Cefaleia</th>
<th>[SIND]</th>
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<th>Mialgias, dores generalizadas</th>
<th>[SIND]</th>
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Tosse ..........................................................................................................................

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<tr>
<th>Dor de garganta, inflamação da mucosa nasal e faríngea, sem sinais respiratórios relevantes</th>
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Dificuldade respiratória .........................................................................................

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<th>Calafrios/Arrepios</th>
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<th>Contacto com doente com gripe</th>
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Legenda: M – Masculino  F – Feminino  S – Sim  N – Não  D - Desconhece

A Preencher pelo INSA

Nº Lab: .................. Nº Req.: ..................

Data: ___/___/___ Nº Amostra: ..................

Pesquisa de vírus Influenza

Resultado: ..............................................

Tomou antivirais durante os últimos 14 dias?

<table>
<thead>
<tr>
<th>Não</th>
<th>Sim, o doente</th>
<th>Sim, um co-habitante</th>
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Nome do antiviral

..........................................................

Vai ser tratado com antiviral? [SIND]

Nome do antiviral

..........................................................
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<tr>
<th>Não pertence à minha lista</th>
<th>Código do caso</th>
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<td></td>
<td>0101_001_2</td>
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**Data da consulta em que este(a) doente foi selecionado**

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**Nesta época (2009/2010), o(a) doente foi vacinado(a) contra a gripe sazonal?**

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<th>S</th>
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**Se sim, vacinado em** (Se não sabe a data exacta, indique a mais aproximada)

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**Qual era o nome da vacina?**

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**O(a) doente foi vacinado contra a gripe sazonal em...**

(Assinalar com X as opções relevantes)

2008/2009
2007/2008

**O(a) doente foi vacinado(a) contra a gripe pandémica?**

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<th>S</th>
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**Se sim, número de doses?**

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**Se sim, recebeu a última dose em** (Se não sabe a data exacta, indique a mais aproximada)

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**O(a) doente alguma vez recebeu a vacina anti-pneumócica?** (injectável)

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**Em que ano?** (última dose)

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<th>Não sabe</th>
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**A doente está grávida?**

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<th>S</th>
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**Se sim em que trimestre está?**

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**O (a) doente tem obesidade mórbida?** ≤10 anos (IMC>25), >10 e <18 anos (IMC>35) e ≥18 (IMC>40)

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<th>S</th>
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**História tabágica do(a) doente** (Assinalar com X a opção relevante):

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<thead>
<tr>
<th>O(a) doente fuma</th>
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<th>Deixou de fumar há mais de um ano</th>
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<th>Nunca fumou</th>
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<th>Não sabe se fuma</th>
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**Doenças crónicas:**

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<th>Diabetes</th>
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<tr>
<th>Doenças cardiovasculares (acidente vascular cerebral, acidente isquémico transitório, enfarte de miocárdio, hipertensão arterial tratada, angioplastia, &quot;by pass&quot; coronário, hipercolesterolemia tratada)</th>
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<th>Insuficiência cardíaca crónica</th>
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<th>Doença renal crónica (falência renal crónica, síndroma nefrótico)</th>
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<th>Doença hepática crónica (cirrose, atriase biliar, hepatite crónica)</th>
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<th>Doença respiratória crónica (asma, bronquite crónica, enfisema, fibrose quística, pneumoconioses, displasia broncopulmonar, fibrose pulmonar)</th>
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<th>Imunodeficiência congênita ou adquirida</th>
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<th>Doença neuromuscular com compromisso da função respiratória</th>
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**Nos últimos 12 meses, quantas vezes foi o(a) doente hospitalizado devido a uma destas doenças crónicas?**

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**Número de consultas de Medicina Geral e Familiar, nos últimos 12 meses.**

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**Quantos anos de escolaridade o(a) doente completou com aproveitamento?**

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**Quantas pessoas vivem na mesma casa com o(a) doente?** (familiares ou não familiares, sem contar com o doente)

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**O(a) doente necessita de ajuda para tomar banho?**

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Annex C – Instruction’s manual
INSTRUÇÕES

O estudo visa estimar a efectividade da vacina contra a gripe sazonal, em indivíduos com idade igual ou superior a 65 anos e da vacina contra a gripe pandémica, em indivíduos de todas as idades.

Este estudo tem um delineamento caso-controlo e está a ser realizado em 2 fases: a primeira, que começou a 15 de Novembro de 2009, para a efectividade da vacina contra a gripe sazonal e a segunda para a efectividade da vacina contra a gripe pandémica que irá começar em Dezembro, a partir do momento em que receber os novos questionários.

Para esta 2ª fase, gostaríamos que seleccionasse casos de síndroma gripal em qualquer idade (2 casos por semana a começar à 2ª feira, com duração até ao final da época gripal, i.e., semana 20 de 2010).

Atenção! Continue a seleccionar os casos de síndroma gripal nos indivíduos com 65 e mais anos (2 casos por semana a começar à 2ª feira, com duração até ao final da época gripal, i.e., semana 20 de 2010) como tem feito até agora.

Por favor devolva os questionários amarelos, mesmo que não tenha seleccionado nenhum caso, assim que receber os novos (verdes).

AVALIAÇÃO DA EFECTIVIDADE DA VACINA DA GRIPE PANDÉMICA
2ª FASE DO ESTUDO

MÉTODO

Pretende-se verificar se há diferenças na percentagem de vacinados entre os 2 grupos seguintes:

1. casos de síndroma gripal com resultado laboratorial positivo para gripe (H1N1)2009;
2. casos de síndroma gripal com resultado laboratorial negativo para gripe (H1N1)2009.

Para a Vigilância da Síndrome Gripal, os exames laboratoriais a efectuar consistem no isolamento e na detecção do RNA do vírus da gripe. Para o isolamento do vírus e a detecção do RNA viral é necessário:

a) Um exsudado da nasofarínge colhido durante os primeiros 5 dias de evolução da doença (de preferência até ao 2º ou 3º dia) em zaragatoa cedida pelo INSA e enviada rapidamente para o INSA pela TNT, transportes expresso.

NOTA PARA MÉDICOS-SENTINELA

Se é Médico-Sentinela e já participa no programa de vigilância clínica e laboratorial da gripe, continue a fazê-lo como habitualmente. A única diferença é que, para este estudo, i.e., para 4 casos de síndroma gripal por semana (2 com idade até 65 anos e 2 com 65 e mais anos), terá de substituir a folha de preenchimento a que está habituado pela folha verde do questionário.
PROCEDIMENTOS

A cada médico participante será fornecido um caderno com instruções, 20 questionários (folhas verdes com frente e verso), e uma folha para a recusa/exclusão de casos para o estudo (folha branca). O questionário deverá ser preenchido sempre que identificar um caso de síndroma gripal na sua lista de utentes ou fora dela.

Note que os questionários estão pré-codificados com o código de caso, no canto superior direito, o que permite a respectiva identificação.

Cada médico receberá também um “kit” para colheita de exsudado nasofaríngeo que deverá também ser identificado com o código de caso (o mesmo que se encontra no canto superior direito do questionário).

1. Selecção dos casos de síndroma gripal

Selecione, na sua lista de utentes, ou fora dela, doentes com síndroma gripal de qualquer idade. Deve identificar 4 casos por semana (2 com idade até 65 anos e 2 com 65 e mais anos). Deve selecionar estes casos a partir de 2ª feira, inclusive, até ao final da época gripal. Se não identificar nenhum caso no dia da semana referido, tente nos dias seguintes, até conseguir. Os casos podem ser seleccionados onde fôr mais conveniente para si, i.e., em consultas, serviços de urgência, serviços de atendimento de gripe, no domicilio, atendimentos complementares, etc.

A definição de síndroma gripal é a recomendada pelo European Center for Prevention and Disease Control (ECDC):

**Grupo A + pelo menos 1 sinal ou sintoma do grupo B + pelo menos 1 sinal ou sintoma do grupo C**

**Grupo A**

- Início súbito (obrigatório)

**Grupo B**

- Febre ou febrícula
- Mal-estar, debilidade, prostração
- Cefaleia
- Mialgias, dores generalizadas

**Grupo C**

- Tosse
- Dor de garganta, inflamação da mucosa nasal e faríngea, sem sinais respiratórios relevantes
- Dificuldade respiratória

Deve excluir do estudo os doentes que:

- Tiveram gripe pandémica confirmada laboratorialmente;
- Vivem num lar ou residência para idosos;
- Têm contra-indicação para a tomada da vacina sazonal ou pandémica.

Nestes casos preencha a folha branca de recusa/exclusão e identifique outro caso de síndroma gripal.
O doente deve tomar conhecimento de que vai ser incluído neste estudo e concordar com essa participação (apenas verbal).

2. Colheita de dados

Preencha o questionário (folha verde, frente e verso) que descreve a síndroma gripal: data de início dos sintomas, sintomas e sinais presentes, estado vacinal em 2009/2010 e em anos anteriores, toma de antivirais, estado de saúde ou doença do indivíduo e gravidez e tempo de gestação, se aplicável. Assinale com um X sobre o espaço ou sobre a letra adequada.

Por favor, confirme directamente com o doente ou no processo clínico as respostas que vai dar.

Destaque o questionário pelo picotado e envie-o juntamente com o exsudado nasofaríngeo cuja colheita se descreve a seguir.

3. Colheita de exsudado da nasofarínge

a) Recolha um exsudado nasofaríngeo de acordo com as instruções seguintes:

1. Introduza a zaragatoa na narina (direita e esquerda) paralelamente ao palato e deixe nessa posição alguns segundos de forma a absorver as secreções;

2. Introduza um pouco mais fundo na mucosa nasal (aproximadamente 2 a 3 centímetros no adulto e até o doente lacrimejar) e rode ligeiramente a zaragatoa;

3. Retire a tampa do tubo de transporte e introduza a zaragatoa para que esta entre em contacto com a esponja existente no fundo do tubo;

4. Pressione fortemente a parte inferior do tubo de modo a que o meio de transporte que embebe a esponja molhe o algodão da zaragatoa;

b) Identifique o tubo com o nome do doente e o código de caso (canto superior direito da folha amarela)

c) A amostra biológica deve ser conservada entre 4 a 8ºC até à recolha pela transportadora, para envio ao laboratório. Salienta-se que o TNT não faz recolhas de sexta a domingo, pelo que as amostras devem ser conservadas nas condições referidas até à recolha, na segunda-feira de manhã.

d) O tubo deve ser acondicionado individualmente, vedando a sua tampa com fita adesiva e introduzindo-o num saco de plástico fechado também com fita adesiva. Este saco deve ser introduzido num envelope almofadado que, por sua vez, deve ser introduzido num saco plastificado identificado com o símbolo TNT.

4. Envio do questionário e zaragatoa para o laboratório

A rapidez no envio das zaragatoas ao laboratório constitui um dos aspectos de maior relevância para a obtenção de resultados válidos no diagnóstico. Neste sentido, solicita-se que sejam enviados o mais brevemente possível.

Para isso, contacte o TNT, que irá entregar os envelopes no Instituto Nacional de Saúde, Dr. Ricardo Jorge. Avº Padre Cruz, 1649-016 LISBOA. Caso não consiga contactar o TNT ligue...
directamente para o Instituto pelo telefone: 217526455, Raquel Guiomar ou 217519220, Anabela Coelho.

Selecione o doente de qualquer idade com síndrome gripal

O doente teve gripe pandémica confirmada laboratorialmente?

✓ O doente cumpre critérios de participação

O doente aceita participar no estudo?

Consentimento informado oral

Preencha o questionário da folha verde (coloque um X sobre o espaço ou letra adequada)

Recolha o exsudado naso-faríngeo (zaragatoa)

Identifique o tubo com o nome do doente e código do caso (acondicionado de acordo com ponto 3 das instruções)

Contacte a empresa de transporte TNT e envie o questionário e zaragatoa para o INSA

Obrigado!