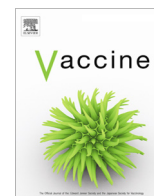


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## Cross-protection to new drifted influenza A(H3) viruses and prevalence of protective antibodies to seasonal influenza, during 2014 in Portugal



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### ABSTRACT

**Introduction:** Immune profile for influenza viruses is highly changeable over time. Serological studies can assess the prevalence of influenza, estimate the risk of infection, highlight asymptomatic infection rate and can also provide data on vaccine coverage. The aims of the study were to evaluate pre-existing cross-protection against influenza A(H3) drift viruses and to assess influenza immunity in the Portuguese population.

**Materials and methods:** We developed a cross-sectional study based on a convenience sample of 626 sera collected during June 2014, covering all age groups, both gender and all administrative health regions of Portugal. Sera antibody titers for seasonal and new A(H3) drift influenza virus were evaluated by hemagglutination inhibition assay (HI). Seroprevalence to each seasonal influenza vaccine strain virus and to the new A(H3) drift circulating strain was estimated by age group, gender and compared with seasonal influenza-like illness (ILI) incidence rates before and after the study period.

**Results:** Our findings suggest that seroprevalences of influenza A(H3) (39.9%; 95% CI: 36.2–43.8) and A(H1)pdm09 (29.7%; 95% CI: 26.3–33.4) antibodies were higher than for influenza B, in line with high ILI incidence rates for A(H3) followed by A(H1)pdm09, during 2013/2014 season. Low pre-existing

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cross-protection against new A(H3) drift viruses were observed in A(H3) seropositive individuals (46%). Both against influenza A(H1)pdm09 and A(H3) seroprotection was highest in younger than 14-years old. Protective antibodies against influenza B were highest in those older than 65 years old, especially for B/Yamagata lineage, 33.3% (95% CI: 25.7–41.9). Women showed a high seroprevalence to influenza, although without statistical significance, when compared to men. A significant decreasing trend in seroprotection from north to south regions of Portugal mainland was observed.

**Conclusions:** Our results emphasize that low seroprotection increases the risk of influenza infection in the following winter season. Seroepidemiological studies can inform policy makers on the need for vaccination and additional preventive measures.

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## 1. Introduction

The pattern of influenza virus circulation is unpredictable and varying between each flu season, thus changing the baseline age specific immunity in the population after each influenza epidemic period. Serological surveillance provides estimates of population immunity level against vaccine preventable diseases [1]. Seroepidemiology data monitor the gradual accumulation of susceptible people, changes in age-specific risk of infection and potential risk of outbreaks [1]. Data from seroepidemiological studies can guide intervention actions concerning vaccination programmes and other preventive measures, especially in high-risk groups. Influenza seroprevalence studies are important contributors to estimate the true incidence and determine the vulnerable populations to disease. Data on population immunity is even more important in pandemic scenarios, being essential to assess pre-existing susceptibility, true infection attack rates, exposure to circulating viruses, estimate asymptomatic infection rates and inform on vaccine coverage [2,3]. Moreover, seroprevalence surveys are the most practical method for accurately estimating the infection attack rate (IAR) in an epidemic such as influenza [4]. However, seroepidemiology data adds important and valuable information to influenza surveillance and could support decision-making in target groups for vaccination, as only a few National Influenza Surveillance Programmes at European level integrate routinely the serological studies in National Influenza Surveillance Systems [5–8]. In Portugal, this is the first seroepidemiological study in the scope of influenza surveillance that aims at assessing the cross-protection to the new drift influenza A(H3) viruses determining the prevalence of seasonal influenza protective antibodies by age, gender and health region as well as considering the relationship with influenza-like illness (ILI) incidence rates observed on seasons before and after the sample collection.

## 2. Materials and methods

To study influenza immunity in the Portuguese population, a non-probabilistic sample was used. Samples were collected from people attending to hospital laboratories for other reasons aside from influenza infection. We developed a cross-sectional study based on a convenience sample of 626 sera collected during June 2014. Sera were selected from all age groups (0–4; 5–14; 15–64 and  $\geq 65$  years old) and both genders, in equal proportion, at 11 hospital laboratories from the Portuguese Laboratory Network for the Diagnosis of Influenza Infection [9], covering all administrative health regions (HR) of Portugal: Norte, Centro, Lisboa e Vale do Tejo, Alentejo, Algarve and including also the Açores (São Miguel and Terceira islands) and Madeira islands. For each HR, an equal representation of all age groups was guaranteed, with the exception of Algarve that didn't select samples from individuals under 5. A remaining volume (minimum selected volume 250  $\mu$ l) of

recently collected sera that comes to laboratory for serological analysis, excluding influenza, were selected for the present study. Sera were randomly selected taking only into account the patient age. Data regarding vaccination or influenza previous infection weren't recorded, because this information weren't available at patient hospital admission registries. All samples were anonymized and data regarding district of residence or sample collection, gender and age were recorded.

The present investigation follows the international ethical guidelines, and was approved by the Health Ethics Committees of the National Institute of Health Dr. Ricardo Jorge (ref. 17/3/2014) and by the Hospital of Divino Espírito Santo of Ponta Delgada (ref. 582/2014).

In all sera, the antibody titers to the influenza virus strains recommended for the tri and quadrivalent vaccines (northern hemisphere, 2014/2015) were assessed: A/California/7/2009 (AH1pdm09), A/Texas/50/2012 (AH3), B/Massachusetts/02/2012 (B/Yamagata lineage), and B/Brisbane/60/2008 (B/Victoria lineage) [10]. Sera from all age groups, with protective antibody titers against A/Texas/50/2012 equal or higher than 80, were tested for the presence of cross reactive antibodies to the new A(H3) drift viruses, antigenically different from vaccine strain: A/Switzerland/9715293/2013 (3C.3a subclade) and A/Hong Kong/5738/2014 (3C.2a subclade) [11]. The selected 150 sera were distributed across all age groups and regions, conforming to the distribution of all A(H3) seropositive samples.

Serum antibody titers were evaluated in duplicate by the technique of hemagglutination inhibition (HI) according to the standard methodology [12] in laboratory biosafety level 2 conditions. Sera were pre-treated with receptor destroying enzyme [RDE (II) "SEIKEN"; Denka Seiken Co. Ltd.] and tested in two fold serial dilutions starting at 1:10 to a final dilution of 1:1280, using guinea pig red blood cells. HI endpoint titer was assessed as the reciprocal of the highest dilution of serum that completely inhibits hemagglutination. HI titer  $\geq 40$  were considered protective against tested virus strain [13] and in titers  $< 10$ , 5 were assigned to enable geometric means titer (GMT) calculation. The WHO Collaborating Centre in London kindly provided the influenza reference virus strains and antisera. Viruses were grown in Mardin Darbin canine kidney (MDCK) cells and in Mardin Darbin Canine Kidney Sialic Acid Over-Expression cells (MDCK-Siat1) [12]. Reference antiserum and homologous virus strains were tested in each assay.

Seroprevalence estimates are presented with respective 95% confidence intervals (95% CI). Differences between groups (gender, age and region) regarding the proportion of sera with antibody titer considered protective (titer  $\geq 40$ ) were tested using the chi-square test, while differences in titers between groups were evaluated using the Kruskal-Wallis test. The level of significance was set at 5%. All statistical analysis was performed in R version 3.0.3.

Seasonal ILI incidence rates were estimated for each age group for 2013/2014 and 2014/2015 influenza seasons using data provided by the Portuguese General Practitioners (GP) sentinel

network (Rede Médicos-Sentinel) that reports the number of ILI cases in a weekly basis since 1990, and is the national ILI incidence rate indicator. As numerator were considered all ILI cases that have met ECDC ILI definition [14] with symptoms onset date falling between week 40/2013 and week 20/2014 or between week 40/2014 and week 20/2015. As denominator was considered the average of population under observation within the GPs sentinel network in the same periods. Given that Portuguese GP sentinel network only collects information on the sample of volunteer GPs and on ILI cases that required medical attendance, it underestimates the true ILI incidence rate and affects the accuracy of estimates.

Seasonal ILI incidence rate by age group were calculated multiplying the proportion of ILI cases positive for each influenza subtype and lineage by seasonal ILI incidence rate to obtain a proxy of seasonal influenza incidence rate by subtype and lineage.

### 3. Results

For the 626 tested samples (from all age groups) highest prevalence of protective antibody titers was detected for A/Texas/50/2012 (39.9%; 95% CI: 36.2–43.8) followed by A/California/7/2009 (29.7%; 95% CI: 26.3–33.4), B/Massachusetts/2/2012 (23.0%; 95% CI: 19.9–26.5) and B/Brisbane/60/2008 (9.1%; 95% CI: 7.1–11.86).

Seroprevalence rates were also reflected by the geometric means titers pattern (Table 1).

#### 3.1. Influenza A(H3) seroprevalence

Seroprevalence of protective antibodies against A/Texas/50/2012 was 60.5% (95% CI: 51.7–68.6) in the age group 5–14 years old, 1.5 times higher than in the general population. It was followed by the elderly ( $\geq 65$  years old) and infants (0–4 years old) with a seroprevalence of 41.3% (95% CI: 33.1–50.0) and 39.3% (95% CI: 30.9–48.4), respectively (Fig. 1). We observed the lowest seroprevalence in adults between 15–64 years old (29.7%; 95% CI: 24.5–35.6). In both influenza seasons the highest A(H3) incidence rates were observed in children between 5–14, 302.1 ILI cases/ $10^5$  inhabitants during 2013/2014 and 577.9 ILI cases/ $10^5$  inhabitants during 2014/2015 (Fig. 1). Individuals over 64 experienced the lowest A(H3) ILI incidence rates in both seasons (101.3 ILI cases/ $10^5$  inhabitants in 2013/2014 and 195.6 ILI cases/ $10^5$  inhabitants in 2014/2015).

#### 3.2. New drift influenza A(H3): A/Hong Kong/5738/2014 and A/Switzerland/9715293/2013

For all samples with a titer  $\geq 80$  to A/Texas/50/2012 ( $n = 150$ ), pre-existing cross protection antibodies titers to A(H3) new drift variants were assessed: A/Hong Kong/5738/2014 (3C.2a subclade)

and A/Switzerland/9715293/2013 (3C.3a subclade), considered antigenically different from vaccine strains and previous A(H3) circulating strains. Protective antibodies titers against A/Hong Kong/5738/2014 and A/Switzerland/9715293/2013 were detected in 46.0% (95% CI: 38.2–54.0) and 46.7% (95% CI: 38.9–54.6) of the sera, respectively. Seroprevalence rates were also reflected by geometric means titers pattern (Fig. 2).

Higher percentages of seropositivity to A(H3) new drift viruses were seen in age groups under 15 and above 64, compatible with seropositivity to the A(H3) vaccine strain, A/Texas/50/2012 (Table 2). It was also evident by GMT values that in different magnitude the younger ( $<15$ ) and older ones ( $>64$ ) were the ones that shown higher antibody titers to the 3 tested A(H3) virus antigens. A higher seroprevalence against A/Hong Kong/5738/2014 was noticed in younger than aged 14; however, to A/Switzerland/9715293/2013 strain the higher seroprevalence was observed in younger than 4 and in adults above 64 years old. Adults between ages 15 and 64 years old showed the lowest rate of protective antibodies to new circulating A(H3) drift strains, 20.0% (95% CI: 10.9–33.8) to A/Hong Kong/5738/2014 and 22.2% (95% CI: 12.5–36.3) to A/Switzerland/9715293/2013 (Table 2).

#### 3.3. Influenza A(H1)pdm09 seroprevalence

Our study showed the highest prevalence of protective antibodies against influenza A(H1)pdm09 (A/California/7/2009) in the age group of 5–14 years old, corresponding to 44.4% (95% CI: 35.9–53.1), 1.5 times higher than in general population (Fig. 1). Lowest seroprevalence against A(H1)pdm09 was found in the elderly (21.4%; 95% CI: 15.2–29.4). During 2013/2014 season A(H1)pdm09 co circulated with influenza A(H3) and B, a high incidence rate for A(H1)pdm09 was observed in children between ages 0 and 4. During 2014/2015 season, the incidence rate for A(H1)pdm09 was very low in all age groups, indicating only a sporadic circulation of this virus subtype [15,16].

#### 3.4. Influenza B seroprevalence

For influenza B, an increase of protective antibodies according to age was observed. In general, higher antibodies titers to B/Massachusetts/2/2012 were found when compared to B/Brisbane/60/2008. Higher seroprevalence to influenza B strains in elderly  $\geq 65$  years old was observed [33.3% (95% CI: 25.7–41.9) to B/Massachusetts/2/2012; 14.3% (95% CI: 9.2–21.5) for B/Brisbane/60/2008]. Lowest level of seroprotection was found to influenza B/Brisbane/60/2008 in all age groups, especially in youngest ones ( $<4$  years old). The seasonal influenza B incidence rate in 2013/2014 was low although during 2014/2015 season influenza B from Yamagata lineage was the predominant circulating viruses. High ILI incidence rates in all age groups were observed, being higher in children between ages 5–14 (770.5 ILI cases/ $10^5$  inhabi-

**Table 1**  
Seroprotection rates against seasonal influenza by hemagglutination inhibition assay (HI titer  $\geq 40$ ) and geometric means titer (GMT) in June 2014 on Portuguese population.

Viruses	Estimation of p	HI $\geq 40$			GMT	
		Number/total	%	95% CI	Value	95% CI
AH1pdm09 <sup>a</sup>		186/626	29.7	26.3–33.4	16.4	15.0–18.0
AH3 <sup>b</sup>		250/626	39.9	36.2–43.8	22.6	20.3–25.3
B_Mass <sup>c</sup>		144/626	23.0	19.9–26.5	13.6	12.6–14.7
B_Brisb <sup>d</sup>		57/626	9.1	7.1–11.6	9.6	9.1–10.2
	<i>p-value</i> <sup>e</sup>	<0.001			<0.001	

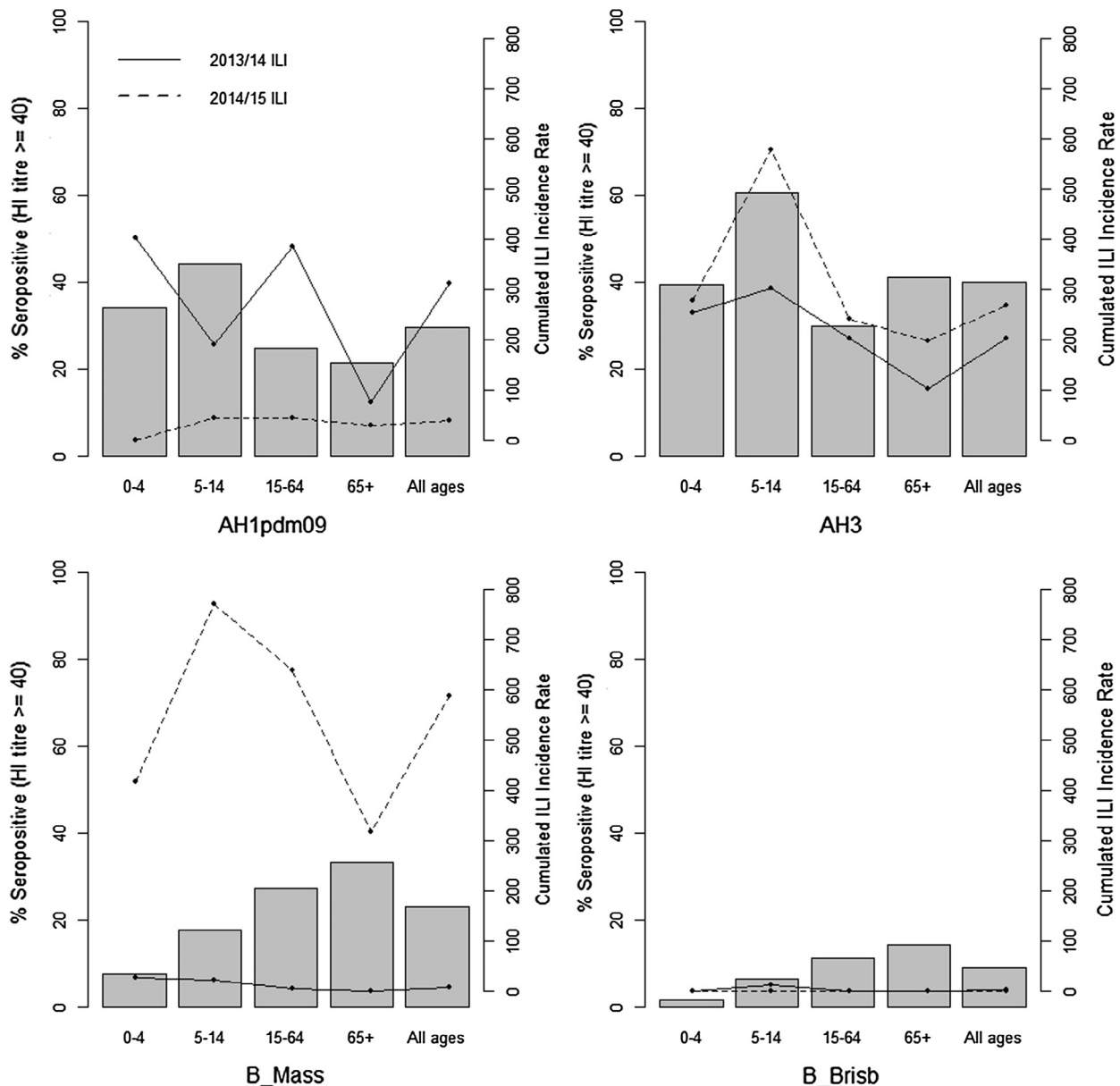
<sup>e</sup> *p*-value refers to the comparison of ratio of viruses (chi-square test), as well as to the comparison of virus titers (Kruskal-Wallis test).

<sup>a</sup> A/California/7/2009.

<sup>b</sup> A/Texas/50/2012.

<sup>c</sup> B/Massachusetts/2/2012 (Yamagata lineage).

<sup>d</sup> B/Brisbane/60/2008 (Victoria lineage).



**Fig. 1.** Prevalence of protective antibodies (titer  $\geq 40$ ) by age group against influenza A/California/7/2009 (AH1pdm09), A/Texas/50/2012 (AH3), influenza B/Massachusetts/2/2012 (B\_Mass) and B/Brisbane/60/2008 (B\_Brisb). Seasonal ILI incidence rate by age group during 2013/2014 and 2014/2015 seasons (GP sentinel Network).

tants). During 2014/2015 season, the lowest incidence rate to influenza B Yamagata was found in individuals over 65 years old (316.6 cases/ $10^5$  inhabitants) (Fig. 1).

### 3.5. Seroprevalence to 2014/2015 vaccine strains

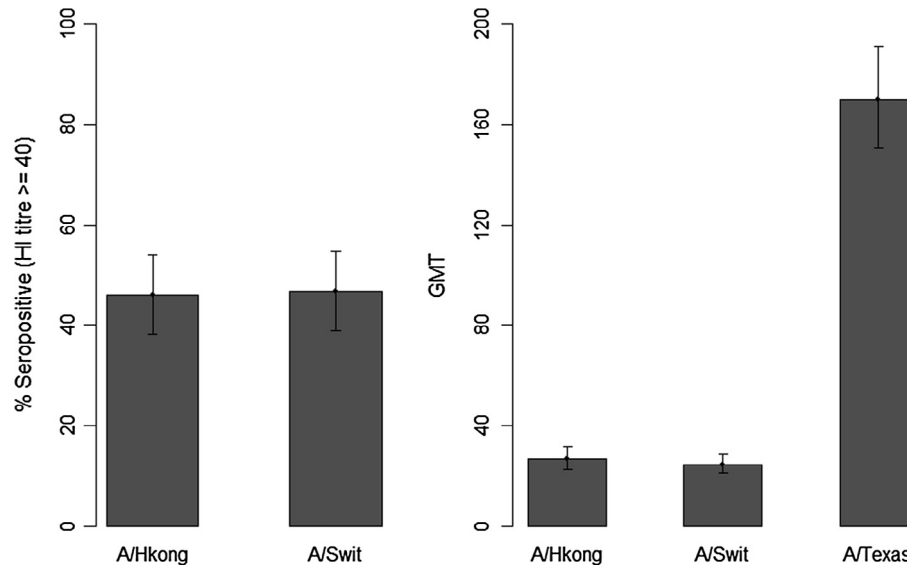
From all tested samples, only 5.8% (95% IC: 4.1–7.9) had HI titers  $\geq 40$  for all the 3 influenza vaccine viruses recommended for 2014/2015 trivalent influenza vaccine [18], indicating a low proportion of individuals with broad protection for seasonal influenza types and subtypes.

### 3.6. Seroprevalence by gender

Analysis by gender showed that seroprevalence rates and GMT to all tested virus strains, including the new drift A(H3), were higher in the females; however, without statistical significance, when compared to seroprevalence and GMT in males (Fig. 3).

### 3.7. Geographical pattern of influenza seroprotection

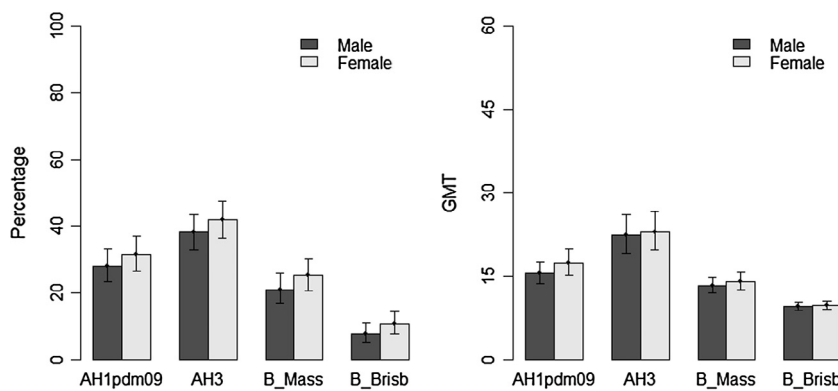
Analysis of seroprotective antibodies in each administrative health region (HR) of Portugal, including the Açores and Madeira islands showed a significant difference between regions. A decreasing trend in seroprotection for influenza, from north to south of Portugal mainland was observed (Fig. 4). The highest seroprevalence rate was observed in the north region to A(H3), 69.4% (95% CI: 59.7–77.6) and the lowest seroprevalence rate, 2.6% (95% CI: 0.5–13.5) in Algarve to influenza B/Victoria lineage. Although Algarve region lack sera from less than 5 years old, this pattern remains the same when children under 5 from all regions are excluded from the analysis. Açores and Madeira regions showed similar seroprotection rates to influenza, although Açores showed higher seroprotection rates for A(H1)pdm09 and influenza B/Yamagata lineage, 41.0% (95% CI: 31.9–50.8) and 35.0% (95% CI: 26.4–44.7), respectively. In Madeira, higher seroprotection rates were observed to A(H3) and B/Victoria, 50.0% (95%



**Fig. 2.** Seroprotection against A/Hong Kong/5738/2014 (A/Hkong) and A/Switzerland/9715293/2013 (A/Swit) in sera with HI titer  $\geq 80$  to A/Texas/50/2012 (n = 150). Geometric mean titers for A/Hkong, A/Swit and A/Texas/50/2012 (A/Texas). Errors bars represent 95% confidence interval.

**Table 2**  
Seroprotection against A/Hong Kong/5738/2014 (A/Hkong), A/Switzerland/9715293/2013 (A/Swit), and A/Texas/50/2012 (A/H3) in sera with HI titer  $\geq 80$  to A/Texas/50/2012, by age group.

Virus	Age group	HI $\geq 40$			GMT	
		Number/total	%	95% CI	Value	95% CI
A/Hkong	0–4	20/31	64.5	46.9–78.9	31.3	22.5–43.6
	5–14	29/48	60.4	46.3–73.0	38.3	29.9–49.0
	15–64	9/45	20.0	10.9–33.8	15.9	12.1–20.8
	65+	11/26	42.3	25.5–61.1	27.5	16.7–45.5
A/Swit	0–4	22/31	71.0	53.4–83.9	40.0	29.8–53.6
	5–14	23/48	47.9	34.5–61.7	25.6	20.4–32.0
	15–64	10/45	22.2	12.5–36.3	14.2	10.7–19.0
	65+	15/26	57.7	38.9–74.5	31.5	20.7–47.8
A/Texas	0–4	31/31	100	–	244.69	190.3–314.6
	5–14	48/48	100	–	163.98	134.1–200.4
	15–64	45/45	100	–	123.14	101.9–148.9
	65+	26/26	100	–	203.39	145.5–284.3



**Fig. 3.** Seroprotection rate against influenza vaccine strains by gender. Sera collected in June 2014 were tested by haemagglutination inhibition assay (HI titer  $\geq 40$ , protective titer) and geometric means titer (GMT) (n = 626). Vaccine strains: A/California/7/2009 (AH1pdm09), A/Texas/50/2012 (AH3), B/Massachusetts/2/2012 (B\_Mass) and B/Brisbane/60/2008 (B\_Brisb). Errors bars represent 95% confidence interval.

CI: 36.4–63.6) and 16.7% (95% CI: 8.7–29.6), compared to Açores. The differences in seroprevalences to influenza in Açores and Madeira were not statistically significant. Seroprevalences

were in accordance with GMT values for each virus and region (Fig. 4).

#### 4. Discussion

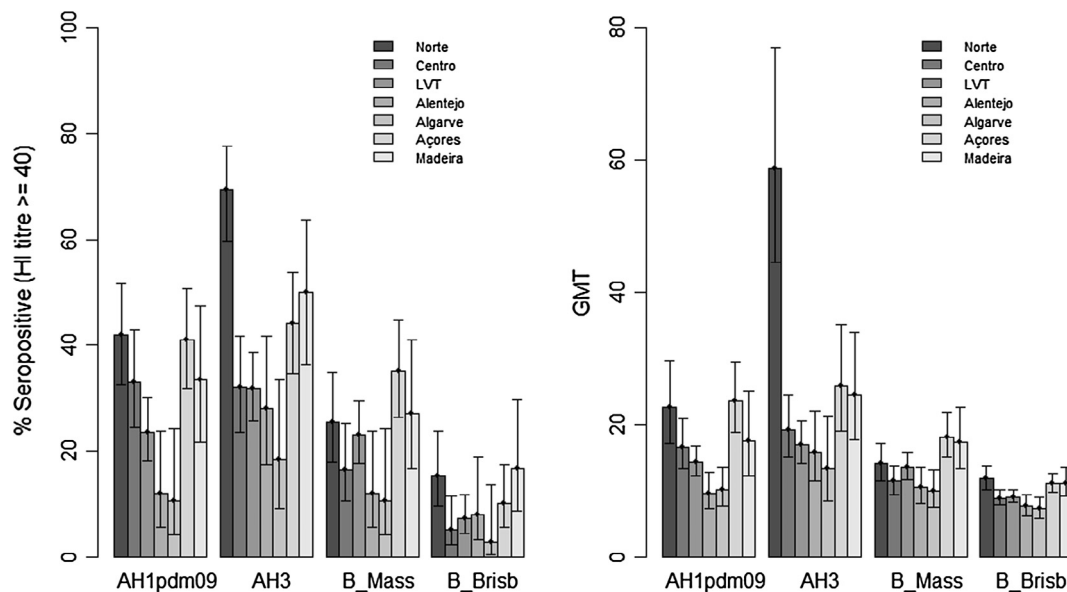
The present study was the first pilot investigation to assess cross-protection and seroprevalence antibodies to influenza in a tentative representative sample of the Portuguese population at the end of 2013/2014 influenza season, in June 2014, interepidemic period. The study showed cross reactive antibodies to new drifted A(H3) viruses and protective antibodies rates to each vaccine influenza virus (sub)type for all age groups, gender and health administrative regions of Portugal.

The seroprotection to each influenza virus type and subtype ranged from 9 to 40% in the studied population. The observed seroprotection was significantly different in each age group. The estimated seroprotection rates against influenza A(H3) and A(H1) pdm09 subtypes were significantly higher compared to influenza B. This observation is in line with predominant circulating viruses in 2013/2014 influenza season, A(H1)pdm09 and A(H3) co-circulated during all winter while influenza B/Yamagata was detected sporadically, and represented only 0.7% of detected influenza viruses [15]. Seroprotection rate against influenza A(H3) virus was higher in children between 5 and 14 years old. This fact is in line with the highest seasonal incidence rate observed in this age group to A(H3) in 2013/2014 season, prior to the study period. Despite this, during 2014/2015, high A(H3) influenza incidence rate was observed in all age groups, especially in children aged 5–14 (577.9 ILI cases/10<sup>5</sup> inhabitants). In fact, 68% (38/56) of analysed A(H3) circulating strains showed antigenic and genetic characteristics dissimilar from the vaccine and earlier circulating strains [16] and were identified as new A(H3) drift variants [17]. Those facts suggest a reduced acquired protection to the new drift A(H3) viruses. In our study, were identified pre-existing cross-reactive antibodies to the new drifted A(H3) viruses in only half of the individuals that showed seroprotection to non-drifted 2014/2015 A(H3) vaccine virus. This finding could explain, together with a limited vaccine effectiveness for A(H3) circulating viruses [10,18], a reduced seroprotection and an elevated ILI incidence rate observed during 2014/2015 influenza season. Seroprotection against new drifted A(H3) viruses was higher in children followed by individuals aged over 64. Adults between 15 and 64,

with lower antibody titers to the new drift A(H3), are more susceptible to infection by these viruses. Also adults showed lower seroprevalence to influenza A, when compared to other age groups, being more likely to be susceptible to influenza A(H1)pdm09 and A(H3) infection [15].

Seroprotection to influenza B is higher in individuals over 15 years old, especially in the elderly ( $\geq 65$  years old), as observed in previous studies [8]. However, seroprevalence to influenza B from Victoria lineage was observed at low levels of protection even in older ones. This fact is related to only sporadic circulation of influenza B Victoria since 2010/2011 winter season [19] and waning immunity that was described in previous studies as less persistent than for influenza A [20]. Influenza B Victoria was detected in circulation in 2010/2011 and since then only influenza B Yamagata was detected during 2012/2013 and 2014/2015. Low number and sporadic cases of influenza B infections were identified during 2011/2012 and 2013/2014 when influenza A predominated. The prevalence of antibodies against influenza B increases with age, for both Yamagata and Victoria lineage, suggesting long-lasting memory immunity for this virus type [21]. Children under 4, born after 2010, were the most vulnerable individuals to influenza B infection showing lower seroprevalence rates. Most of these children had only been exposed once to influenza B Yamagata, before sample collection (July 2014), as this virus only circulated in high level during 2012/2013 winter season, in line with the lowest seroprevalence detected in younger ones in the present study. Previous studies described that detectable antibodies to influenza in children gradually increase with age, approximately 60–70% of all children up to the age of 12 were serologically naïve and have to be considered susceptible to influenza B, such being detected in more than 90% of children only at age of 18 years [8].

The low proportion of samples with protective antibodies against the three vaccine strains (5.8%) is in line with the low vaccine coverage estimates for 2013/14 season in the general population and elderly (17.1% and 49.9%, respectively) [22]. But given that our study was set up during an interepidemic period, it could also be linked to the waning of vaccine protection observed a few months after vaccination [23].



**Fig. 4.** Seroprotection rate against influenza by haemagglutination inhibition assay (HI titre  $\geq 40$ ) and geometric means titer (GMT) by administrative health region, in sera collected in June 2014, Portugal (n = 626). Vaccine strains: A/California/7/2009 (AH1pdm09), A/Texas/50/2012 (AH3), B/Massachusetts/2/2012 (B\_Mass) and B/Brisbane/60/2008 (B\_Brisb). Errors bars represent 95% confidence interval.

However, without statistical significance, women showed higher seroprevalence to influenza, as shown by a previous study [8]. This immune response might be linked to hormones and genetic sex-based differences that interfere with antibody production by both genders [24]. It should also be considered that women are the ones that usually take care of the children which allows a more direct contact and could favour the transmission of influenza virus by the children, one of the groups with high influenza incidence.

Across Portugal, higher seroprotection rates were identified in the north health administrative region with a decreasing trend to the south of Portugal mainland. In the scope of the National Influenza Surveillance Program the north region accounted for the highest percentage of influenza A detected viruses, during 2013/2014 season, in correlations with serological findings [15].

We acknowledge some limitations in our serological study. Information regarding neither influenza vaccination nor influenza previous infection during 2013/2014 season were collected for each patient, limiting the interpretation of sources for acquired immunity to influenza, thus making it impossible to differentiate between seropositivity due to natural infection or immunization. Titer of 1:40 that correlates with a 50% reduction of the risk of infection was assumed for all age groups although previous studies suggest a higher HI cut off titer for children [25,26]. It should also be mentioned that detected antibodies by HI does not fully correspond to all functional antibodies [26], and cellular immunity was not evaluated, although it is also related to protection [5,13].

There are some limitations recognized about the sampling because a non-probabilistic sample was used, however samples were collected during a period without influenza circulation in the community, June 2014 [27] and selected in hospitals from the Portuguese Laboratory Network, covering all administrative health regions, Portugal mainland and the Açores and Madeira Islands. Cross-protection against the new drift A(H3) influenza virus was evaluated in a sample of sera positive for non-drifted virus, with HI titer  $\geq 80$ , adding some imprecision to the calculated seroprevalence for A(H3) new drift strains. Nevertheless, this sample was distributed across all study population and covered all age groups with a distribution similar to the overall A(H3) positive samples. To determine seroprotection to influenza B was not performed ether-treatment of the influenza B antigens for HI assay, prioritizing increased specificity at the expense of sensitivity [28–31]. Study seroprevalence results showed consistency with the viruses that circulated before and after the study, although it should be assumed caution has prevalence of influenza B seroprotection could be underestimated. The sample size and previous data on ILI incidence rates by age group only enabled the analysis of four age group categories, resulting in a broad age group, especially in adults aged 15–64 disallowing evidence patterns within subgroups of this population. In future serosurvey studies and in influenza surveillance program will be collected the date of birth for each ILI case and will be increased the number of sera selected for serological evaluation to enable desegregation of considered age groups. A single-year observation imposes some limitation on data interpretation. To have antibody prevalence data before and after the influenza annual epidemic the authors plan to repeat this serosurvey annually to track the effects of the outbreaks in the population seroprevalence and immunity.

## 5. Conclusions

The main strength of our findings is the close relation of seroprevalence and the dominant influenza virus in previous and following influenza season. Overall, our results suggested that at the end of 2013/2014 season, the Portuguese population was more

susceptible to influenza B, in particular the younger ones, consistent with a predominant circulation of influenza B in the winter following the study, 2014/2015 influenza season. However, this should be interpreted with caution due to a possible underestimated seroprevalence to influenza B. The higher ILI incidence rates in 5–14 age group, highlights the potential of this group to spread influenza in the community. Also evident for influenza B is the importance of a memory immunity in older individuals. Limited pre-existing cross reactive antibodies to the new drift A(H3) strains in the general population were observed. Only half of seropositives to influenza A(H3) vaccine strain showed seroprotection to the new drift A(H3) strains. This is in correlation with the high A(H3) incidence rates during 2014/2015 season, in spite of the high A(H3) seroprevalence observed in 2014. A low proportion of all population had broad protection to all seasonal influenza viruses, thus suggesting low vaccine uptake, and the need for intervention and recommendations on preventive measures.

In the future, serology studies should be taken in the scope of influenza surveillance programmes to better understand the dynamic of influenza transmission, seroprotection in population and establish an annual immunological profile to identify most vulnerable age groups for influenza infection in each winter season.

## Authors' contributions

R. Guiomar, P. Pechirra, S. Pereira da Silva, B. Nunes performed the conception and design of the study and interpretation of data; All authors were involved in the acquisition of data; R. Guiomar, P. Conde, P. Cristóvão, A.C. Maia, performed laboratory analysis; S. Pereira da Silva, B. Nunes performed statistical analysis; R. Guiomar, P. Pechirra, S. Pereira da Silva, Ana Paula Rodrigues, B. Nunes, Maria João Peres and L. Mota-Vieira were involved in drafting the article and revising it critically for important intellectual content; All authors had performed final approval of the version to be submitted.

A. Fernandes, J. Pereira-Vaz, S. Almeida, R. Côrte-Real, M.J. Peres, G. Andrade, L. Mota-Vieira, F. Caldeira, J. Bruges-Armas, J.T. Guimarães, M. Cunha, coordinated the study at Hospital level, where the serum samples were selected.

The authors have agreed to the manuscript. The network members were informed of the submission and were asked for disclosure.

## Conflict of interest

All the authors declare that they have no competing interests.

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