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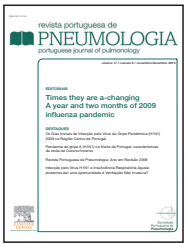

ORIGINAL ARTICLE

The early days of pandemic (H1N1) 2009 virus infection in the central region of Portugal

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Abstract
Background: The first case of pandemic (H1N1) 2009 virus infection was diagnosed in the central region of Portugal on June 16, 2009, in a woman infected in Canada.
Methods: The aim of our study was, first to characterize the clinical and epidemiologic aspects of all the patients with clinical manifestations included in the definition of a *case for investigation* with samples submitted to diagnosis of the pandemic (H1N1) 2009 virus infection, in the central region of Portugal; second, to assess the precision of the case definition of *case for investigation* considered in the study according to the presence or the absence of fever at the moment of clinical observation. We reviewed the medical records of all the patients presenting with Influenza like-illness classified as a *case for investigation* and the first cases of patients infected with the new pandemic (H1N1) 2009 virus, diagnosed in the central region of Portugal during the pandemic period between June and August, 2009, were analyzed. Real-time

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reverse-transcriptase polymerase-chain-reaction (RT-PCR) testing was used to confirm the pandemic (H1N1) 2009 virus infection. Data collection was performed using a standardized paper format in agreement with the General Health Directorate.
Results and discussion: Pandemic (H1N1) 2009 virus infection was confirmed in 255 patients. Overall, median age was 23 years and 42.7% were included in the category of 20 to 29 years. Confirmed infection in patients with less than 2 years or greater than 50 years was a rare event. The first cases were imported from Europe, namely France, Spain and England. In a second phase, pandemic (H1N1) 2009 virus infection was acquired in the south of Portugal (Algarve). The incidence rate for pandemic (H1N1) 2009 virus infection was 10.7 per 100,000 persons and was different according to the district. It was higher in the district of Coimbra and Guarda where the main roads are connecting to Europe. The median calculated incubation period for the for pandemic (H1N1) 2009 virus infection was 2 days. The length of the clinical manifestations until the patients sough for medical observation had a median time of 2 days. All the cases were of mild to moderate severity. No deaths were observed.
Conclusions: The early days of pandemic (H1N1) 2009 virus infection were characterized by cases of disease of mild to moderate severity, in our region. Most affected patients were young adults, with the extreme ages of life being spared. An early diagnosis, strict isolation and treatment procedures could have slowed the spread of the infection.
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PALAVRAS-CHAVE
Gripe, influenza;
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Os dias iniciais da infecção pelo vírus da gripe pandémica (H1N1) 2009 na região centro de Portugal

Resumo
Introdução: O primeiro caso de infecção pelo vírus da gripe pandémica (H1N1) 2009, foi diagnosticado na região centro de Portugal no dia 16 de Junho de 2009, numa mulher infectada no Canadá.
Métodos: O nosso estudo tem por objectivos, em primeiro lugar caracterizar os aspectos clínicos e epidemiológicos de todos os doentes que tiveram manifestações clínicas incluídas na definição de caso para investigação com amostras submetidas para diagnóstico da infecção pelo vírus da gripe pandémica (H1N1) 2009; em segundo lugar, avaliar a precisão da definição de caso para investigação de acordo com a presença ou ausência de febre no momento da observação clínica. Efectuámos a revisão dos registos médicos de todos os doentes classificados como caso para investigação e analisaram-se os primeiros casos de doentes infectados com o novo vírus da gripe pandémica (H1N1) 2009, diagnosticados na região centro de Portugal durante o período pandémico compreendido entre Junho e Agosto de 2009. Foi utilizado o método da reacção em cadeia da polimerase de retrotranscripção em tempo real para confirmação da infecção pelo vírus da gripe pandémica (H1N1) 2009. A colheita de dados foi efectuada de forma padronizada em suporte de papel de acordo com as normas da Direcção Geral de Saúde.
Resultados e discussão: A infecção pelo vírus da gripe pandémica (H1N1) 2009, foi confirmada laboratorialmente em 255 casos. A idade média foi de 23 anos e 42,7% foram incluídos na categoria dos 20 aos 29 anos. A infecção em doentes com menos de 2 anos ou mais de 50 anos foi um acontecimento raro. Os primeiros casos foram importados da Europa: França, Espanha e Inglaterra. Numa segunda fase, a infecção foi adquirida no sul de Portugal (Algarve). A taxa de incidência de infecção pelo vírus da gripe pandémica (H1N1) 2009 foi de 10,7 por 100 000 pessoas e foi diferente consoante o distrito. Foi mais elevado no distrito de Coimbra e da Guarda onde estão as principais estradas de conexão com a Europa. O período de incubação calculado para a infecção pelo vírus da gripe pandémica (H1N1) 2009 foi de 2 dias. A duração das manifestações clínicas até os doentes procurarem observação médica teve um valor mediano de 2 dias. Todos os casos foram de gravidade ligeira a moderada, sem casos de morte.
Conclusões: Os dias iniciais da infecção pelo vírus da gripe pandémica (H1N1) 2009, foram caracterizados por casos de doença com gravidade ligeira a moderada, na nossa região. Os mais afectados foram os jovens adultos, com as idades extremas da vida poupadas. O diagnóstico precoce, o isolamento estrito e o tratamento podem ter diminuído a disseminação da infecção.
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Introduction

At the beginning of April 2009, several outbreaks of influenza-like illness were identified in Mexico in different regions of the country.[1]

Later on April 24th, of the same year, the Centers for Disease Control and Prevention (CDC) described the first confirmed cases of swine-origin influenza A (H1N1) virus infection in the states of Texas and California,[2] of the United States of North America and documented in Mexico the dissemination within humans of a virus similar, in genetic terms, to the influenza A viruses not previously identified among swine or humans.[3,4]

Geographical spread occurred in an exceptionally rapid way so that between April 29th and June 11th, 2009, the World Health Organization (WHO) reported laboratory confirmed cases of infection in 74 countries and territories in more than two WHO regions. This global spread led WHO to announce that a pandemic was underway.[5]

In Portugal, the National Influenza Preparedness Plan was ready since March 2006, and was activated in April 2009 by the Portuguese Heath Directorate (DGS).[6]

The first case of pandemic (H1N1) 2009 virus infection in our country was diagnosed on the 29th of April, 2009, in a woman with epidemiologic link to Mexico (Cancun).

This article aims, in the first place to describe the clinical and epidemiologic features of all the patients that had clinical manifestations included in the definition of *case for investigation* with samples submitted for diagnosis of the pandemic (H1N1) 2009 virus infection, with special emphasis on the first 255 patients infected with the new pandemic (H1N1) 2009 virus, diagnosed in the central region of Portugal between June and August 2009; in the second place to assess the precision of the definition of *case for investigation* according to the presence or absence of fever at the moment of the clinical observation.

Methods

Data sources

A national network of laboratories was set up to monitor pandemic (H1N1) 2009 virus infection in Portugal, organized under the auspices of the ministry of health in collaboration with regional health administration authorities and the National Institute of Health.

Clinical services included health care centers at the level of the primary health care, but also community and teaching hospitals all over the country with dedicated facilities to attend patients with suspected cases of pandemic (H1N1) 2009 virus infection.

The guidelines "Orientações Técnicas da Direcção-Geral da Saúde (DGS)" were used for surveillance, diagnosis, reporting, prophylaxis and treatment.

A *case for investigation* was understood as a patient with the following symptoms: sudden onset of fever (temperature $\geq$ 38,0 °C) or history of fever in the last days and, at least, two of the following symptoms: cough, headache, sore throat, myalgia/arthralgia, rhinorrhea, vomiting/diarrhea and/or severe acute respiratory disease (including pneumonia) suggesting an infectious etiology.[7]

Laboratory diagnosis of pandemic (H1N1) 2009 virus infection was done by a real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay in agreement with the protocol established by the Centers for Disease Control (CDC)[8] and World Health Organization (WHO) involving university hospitals and regional health administration authorities organized in a laboratory network working in collaboration with the National Institute of Public Health, in Lisbon.

A confirmed case was defined as one in agreement with the definition of *case for investigation* and with a positive result of a RT-PCR assay performed at a laboratory of the reference network.

Results were available in less than 24 hours after the samples arrived at the laboratory.

Oropharyngeal and nasopharyngeal swabs were collected at presentation in each clinical service and sent in viral transport medium refrigerated at 4 °C to the closest reference laboratory.

Patients classified as cases for investigation were admitted to an infectious disease service where they could be treated, and maintained under restricted respiratory isolation procedures while waiting for the RT-PCR assay.

The decision to discharge the patients was based on the existence of a negative result on the testing of combined oropharyngeal and nasopharyngeal samples, a return to normal body temperature on two consecutive days and the absence of respiratory symptoms.

A national call center (Linha Saúde 24) was set up to attend patients with influenza-like illness and to refer them to the closest available place with dedicated service for pandemic influenza.

Data was collected using an adopted standardized paper form in agreement with the guideline "Orientação Técnica" (Gripe OT-3) from the General Health Directorate. They were recorded on case report forms for standardized data collection where demographic data, symptoms, place and name of the attending physician and, information concerning the past seven days before the beginning of the symptoms namely about a stay or residence in a area where there has been registry of transmission of influenza in the community (when, where and information about the places or countries visited), about history of close contact with a probable or confirmed case of infection with pandemic (H1N1) 2009 virus and about work in a laboratory that processed biological samples with influenza virus.

We highlight, that the registry paper format for the observation of the patients (Gripe OT-3) besides listing the symptoms included in the case definition, also allowed the registry of additional clinical elements that included: dyspnea, epistaxis, sneezing, nausea, conjunctivitis and others.

All this information (Gripe OT-3) was sent with the samples to the laboratory and to the central and regional public health authorities.

Fourteen health care centers with specific attending services for pandemic (H1N1) 2009 virus infection at the primary health care level and ten hospitals in the central region of Portugal were involved in the emergency public health response. The laboratory of virology of the University Hospital of Coimbra E.P.E. was the reference laboratory for the central region.

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Data analysis was done in collaboration with University Hospital of Coimbra E.P.E. and Center Regional Health Administration (Departamento de Saúde Pública e Planeamento) representatives.

Confidentiality of the patients was maintained. Approval by the review boards of the participating institutions was not required because the collection and analysis of data about the pandemic (H1N1) 2009 virus infection was considered of public health interest.

Statistical analysis

Variables were summarized as means and standard deviation or as median and interquartile range.

For categorical variables, the percentages of patients in each category were calculated.

The clinical features were compared between subgroups of patients infected versus non infected with the virus H1N1 infection using the Student's t-test, chi-square test, or Fisher's exact test, as indicated.

A multiple logistic-regression model was built considering the significant variables ($P < .05$) on the bivariate analysis to identify independent predictive factors associated with pandemic (H1N1) 2009 virus infection.

For the multivariate analysis, the p-value of the model, validity index and area under the curve (AUC) of the receiver operating characteristic (ROC) model was calculated.

A 2-sided *P*-value of less than 0.05 was considered to indicate statistical significance. All statistical analysis was carried out with the use of SPSS software (Statistical Package for Social Sciences) (release 16).

Results

Demographic characteristics

We reviewed the medical records (Gripe OT-3) of all the patients with samples submitted for diagnostic testing procedures for pandemic (H1N1) 2009 virus.

For the central region of Portugal a population of 2.383.284 inhabitants was considered, distributed by 6 districts.[9]

We considered for our analysis the period that started on June 16 with the diagnosis of the first laboratory-confirmed infection with pandemic (H1N1) 2009 virus in our region and ended on August 28, 2009.

During this period, samples from all the patients with influenza-like illness included in the definition of *case for investigation* were collected and tested in a consecutive way.

Samples from 828 patients were processed and submitted for diagnosis of pandemic (H1N1) 2009 virus, by RT-PCR.

The overall median age of the patients was 23 years (IQR, 13-35 years; range, 21 days to 88 years) (Table 1). However, patients with laboratory-confirmed pandemic (H1N1) 2009 virus infection had a lower median age [21 years (IQR, 16-27; range, 1 month to 84 years)].

Most of the patients (56%) were male. Distribution by sex and pandemic H1N1 virus infection, showed no significant association ($P = .379$).

A significant ($P < .001$) association was found between the distribution by age categories and the distribution by pandemic (H1N1) 2009 virus infection.

The category of the 20 to 29 years included 42.7% of the pandemic (H1N1) 2009 virus infected patients and the category of the patients with less than 10 years included 21.8% of the patients classified as *case for investigation* without laboratory confirmation that is, non-infected patients with the pandemic strain ($P < .001$).

A small proportion of patients (11%) with pandemic (H1N1) 2009 virus infection were more than 40 years old, and 12.2% were less than 10 years. Children with less than 2 years represented 2.7% of the total amount of patients with confirmed infection.

The overall incidence of influenza-like illness, classified as case for investigation, was 34.7 per 100,000 persons. However, the incidence rate of pandemic (H1N1) 2009 virus infection was 10.7 per 100,000 persons.

Age-adjusted rates, varied from 2 cases per 100,000 persons in the group of individuals older than 50 years to 35 cases in the group 20-29 years of age (Table 2).

The proportion of non-infected patients by pandemic (H1N1) 2009 virus was greater in all but one category of 20 to 29 years, where rate between the proportion of infected versus non-infected was 1:1.

Origin and geographic distribution of the patients

The first case of confirmed pandemic (H1N1) 2009 virus infection in our region was diagnosed on June 16, 2009, in a patient coming from Canada (Montreal).

Only two additional cases were diagnosed that month. The first domestic case—in a patient without a clear history of exposure, was diagnosed on August 13. Until August 28, 2009, 255 cases of pandemic (H1N1) 2009 virus infection were diagnosed with a sharp drop thereafter.

Most of the patients were observed at dedicated areas for observation of patients with fever and suspicions of infection, namely at the University Hospital E.P.E. (35%) and at Children's Hospital (16%), both in Coimbra. Other patients were observed at centers with dedicated flu attendance places at the primary health care level.

Most of the patients (66.5%) reported an epidemiologic link with an individual with a suspected or confirmed pandemic (H1N1) 2009 virus infection, however, only 33.2% of these patients with influenza-like illness were laboratory-confirmed with pandemic (H1N1) 2009 virus infection.

Of those patients without any description of an epidemiologic link to a suspected case of influenza, 26% presented with confirmed infection by the pandemic (H1N1) 2009 virus infection.

For the patients with laboratory-confirmed pandemic (H1N1) 2009 virus infection, the great majority (72%) reported the existence of a contact with a positive case while this happened in only 28% of the negative cases ($P = .030$).

Two patterns of spread of the infection were observed in the central region of Portugal.

The first cases of pandemic (H1N1) 2009 virus infection, were imported from outside the country, namely from Spain.

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Table 1 Demographic characteristics

	Total (n = 828)		(H1N1) 2009				*P*-value	Odds ratio
			Negative (n = 573)		Positive (n = 255)			(95% confidence interval%)
Sex, n (%)								
Female (Ref.)	363	43.8%	257	44.9%	106	41.6%	.379[a]	1,143 [0.848; 1,541]
Male	465	56.2%	316	55.1%	149	58.4%		
Total	828	100.0%	573	100.0%	255	100.0%		
Age,years								
No.	828	573	255	.037[b]	0.989[c] [0.980; 0,998]			
Mean	25.39	26.37	23.18					
Median	23.00	25.00	21.00					
Standard deviation	16.97	18.32	13.22					
Minimum	0.06	0.06	0.08					
Maximum	88	88	84.00					
1st quartil	13	11	16.00					
3rd quartil	35	38	27					
< 2 yr	41	5.0%	34	5.9%	7	2.7%	< .001[a]	
0-9 yr	156	18.8%	125	21.8%	31	12.2%	< .001[a]	
10-19 yr	156	18.8%	95	16.6%	61	23.9%		
20-29 yr	228	27.5%	119	20.8%	109	42.7%		
30-39 yr	131	15.8%	105	18.3%	26	10.2%		
40-49 yr	79	9.5%	65	11.3%	14	5.5%		
> 50 yr	78	9.4%	64	11.2%	14	5.5%		

(*) Chi-square test.
(**) Mann-Whitney test.
Ref.: Category versus which comparison are made.
a) Increased risk per year of age.

Table 2 Age-specific incidence rates for pandemic (H1N1) 2009 virus infection

Age, years	No. of total population, % (n = 2,383,284)	No. of patients with H1N1 virus infection, % (n = 255)	Incidence rate, no. of cases per 100,000 population
0-9	**218,076 (9.2)**	31 (12.2)	14.2
10-19	**238,780 (10.0)**	61 (23.9)	25.5
20-29	**310,450 (13.0)**	109 (42.7)	35.1
30-39	**351,796 (14.8)**	26 (10.2)	7.4
40-49	**339,135 (14.2)**	14 (5.5)	4.1
> 50	**925,047 (38.8)**	14 (5.5)	1.5
Overall	**2,383,284 (100)**	255 (100)	10.7

In a second phase, half of the cases were most probably acquired in the south of Portugal (Algarve).

The incidence rate of the novel pandemic (H1N1) 2009 virus infection, varied according to the geographic location (Figure).

During the period included in our study (June 16 to August 28), the incidence rate was greater but similar in the two districts connecting the interior of the Iberian peninsula to the Atlantic ocean—Coimbra (17.2 per 100,000 persons) and Guarda (19.4 per 100,000 persons).

Manifestations and clinical course

Overall, 86% of the patients had fever (Table 3), that was more common (91%) in patients with pandemic (H1N1) 2009 virus infection than in negative patients (84%), ($P = .009$).

We could analyze that fever appeared simultaneously with other symptoms in 66% of the cases and that in 31% of the cases fever appeared after other manifestations, both in patients with a positive test as in cases negative for pandemic (H1N1) 2009 virus ($P > .05$).

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Figure 1 Distribution of the incidence rates, by district, of the confirmed cases of human infection with pandemic (H1N1) 2009 virus, in the central region of Portugal. Period between June 16, and August 28, 2009.
Numbers indicate the incidence rate in each district.

Other commonly reported signs and symptoms, included: myalgia (67%), cough (61%), headache (59%), sore throat (52%), rhinorrhea (32%), vomiting (16.6%) and diarrhea (21%).

Bivariate analysis shown that pandemic (H1N1) 2009 virus infected patients were more likely to have fever (90.6% versus 83.7%, $P < .009$), cough (81.6% versus 51.7%, $P < .001$), headache (64,3% versus 56.6%, $P < .038$), rhinorrhea (37.3%, versus 29.4%, $P < .0025$), and sneezing (20.0% versus 11.5%, $P < .001$). However, they were less likely to have dyspnea, vomiting and diarrhea than patients with a negative result for pandemic (H1N1) 2009 virus.

Also, patients with infection with pandemic (H1N1) 2009 virus had a greater median number of symptoms (5 symptoms versus 4, $P < .001$).

The assessment of the severity of the clinical case was done by the physician that observed the patient with a *case for investigation* on the first appointment and the majority (80%) of the cases, were considered of light to moderate severity.

During the period considered in our study, no patients had pneumonia and no cases were admitted to the intensive care units.

With the aim to calculate the duration of the disease of the cases validated as a case for investigation, all of the patients that in our database presented a registry with the date of the beginning of its symptoms (n = 819) were included and we calculated the duration of the interval between the start of the manifestations and the date of the medical observation. The median length of time until medical observation was 2 days (range, from less than a day to 13 days).

We calculated the approximate period of incubation, taking into consideration only the cases with confirmed pandemic (H1N1) 2009 virus infection, with accurate known date of the last contact with a suspicious case or with an epidemiologic link to a country or place with active infection circulating in the community. The period of incubation pandemic (H1N1) 2009 virus, had a median duration of 2 days (range, 1 to 8 days).

Factors associated with pandemic (H1N1) 2009 virus infection

A multiple logistic regression analysis model (Table 4) was built up considering the covariates with significant ($P < .1$) association in the bivariate analysis that were the following: age, cough, headache, dyspnea, epistaxis, fever, myalgia, nausea, vomiting, diarrhea and number of symptoms.

An optimized model (greatest p-value considered for backward regression) was built up, that showed the existence of the following independent risk factors for pandemic (H1N1) 2009 virus infection: age (odds ratio, 0.982; 95% confidence interval [CI], 0.971 to 0.993; $P = .001$), cough (odds ratio, 4.450; 95% confidence interval [CI], 3.082 to 6.425; $P < .001$), headache (odds ratio, 1.451; 95% confidence interval [CI], 1.024 to 2.057; $P = .036$), dyspnea (odds ratio, 0.418; 95% confidence interval [CI], 0.198 to 0.881; $P = .022$), epistaxis (odds ratio, 3.855; 95% confidence interval [CI], 1.034 to 14.372; $P = .044$), fever (odds ratio, 1.840; 95% confidence interval [CI], 1.106 to 3.060; $P = .019$), myalgia (odds ratio, 1.491; 95% confidence interval [CI], 1.014 to 2.194; $P = .042$) and vomiting (odds ratio, 0.463; 95% confidence interval [CI], 0.288 to 0.744; $P = .001$).

The optimized model showed that younger people had a greater probability of having laboratory-confirmed pandemic (H1N1) 2009 virus infection.

Some symptoms were positively correlated with the probability of having pandemic (H1N1) 2009 virus infection,

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Table 3 Symptoms, contact and epidemiologic link

	Total (n = 828)		(H1N1) 2009				*P*-value
			Non confirmed H1N1 (n = 573)		Confirmed (n = 255)		
	n	(%)	n	(%)	n	(%)	
Cough	504	60.9%	296	51.7%	208	81.6%	< .001[a]
Headache	488	59.0%	324	56.6%	164	64.3%	.038[a]
Sore throat	431	52.1%	294	51.4%	137	53.7%	.536[a]
Rhinorrhea	263	31.8%	168	29.4%	95	37.3%	.025[a]
Dyspnea	56	6.8%	46	8.0%	10	3.9%	.029[a]
Epistaxis	12	1.5%	5	0.9%	7	2.7%	.055[b]
Sneezing	117	14.1%	66	11.5%	51	20.0%	.001[a]
Fever	710	85.9%	479	83.7%	231	90.6%	.009[a]
Myalgia	552	66.7%	370	64.7%	182	71.4%	.059[a]
Arthralgia	176	21.3%	114	19.9%	62	24.3%	.155[a]
Vomiting	137	16.6%	109	19.1%	28	11.0%	.004[a]
Diarrhea	173	20.9%	133	23.3%	40	15.7%	.014[a]
Nausea	94	11.4%	73	12.8%	21	8.2%	.058[a]
Conjunctivitis	34	4.1%	22	3.8%	12	4.7%	.565[a]
Contact[c]	148	37.9%	64	25.9%	84	58.7%	< .001[a]
Link[d]	550	66.4%	367	64.0%	183	71.8%	0.030[a]

[a]Chi-square test.
[b]Fisher's exact test.
[c]Contact: close contact with a probable or confirmed case of pandemic (H1N1) 2009 virus infection, during the 7 days prior to the beginning of symptoms.
[d]Link or epidemiologic link: existence of a stay or residence in a area where there was registry of transmission in the community of pandemic (H1N1) 2009 virus infection, during the 7 days prior to the beginning of symptoms.

namely cough (4.5 times), epistaxis (3.9 times), lack of dyspnea (2.4 times), vomiting (2.2 times), fever (1.8 times), headache (1.5 times), and myalgia (1.5 times),

The *p* value and the area under curve of the receiver operating characteristic (ROC) proved the model to be adequate to explain the presence of laboratory-confirmed pandemic (H1N1) 2009 virus infection.

The sensitivity, specificity, positive predictive value and the negative predictive value of the definition of *case for investigation* were determined, having as a comparision the RT-PCR results and the presence or absence of fever (confirmed) on the date of the clinical observation (Table 5).

The sensitivity of the definition of *case for investigation* associated to the presence of fever confirmed in the date of observation was 91% for the identification of the presence of pandemic (H1N1) 2009 virus.

The specificity of the definition of a *case for investigation* associated to the absence of fever confirmed in the date of observation was 16% for the identification of absence of pandemic (H1N1) 2009 virus.

The positive predictive value was 33% and the negative predictive value was 75%, this means that the probability of an individual with fever at the moment of clinical observation to have, in fact, pandemic (H1N1) 2009 virus infection was lower than the probability of an individual without fever not having pandemic (H1N1) 2009 virus infection.

Discussion

We described the first group of patients infected with the pandemic (H1N1) 2009 virus, diagnosed between June and August, 2009, in the central region of Portugal during the early days of the pandemic.

All the patients were admitted to hospitals in agreement to strict isolation procedures, and submitted to quarantine as recommended during this period.

In our region, the first cases were diagnosed among travelers and there was a delay of almost two months between the diagnosis of the first imported case (June 16) and the diagnosis of the first domestic case (August 13). In other countries and regions there was a more explosive spread.[10-12]

Possible explanations could be less crowded living conditions, early detection, good isolation practices and intensive public health measures implemented in our region. All patients were quarantined during this period and no nosocomial cases of infection were described.

Our data suggest that in the early phase of the pandemic, most of the cases were imported and entered in our region by the roadways that allow the displacement of people coming from France and Spain where at the same time occurred active dissemination of the pandemic (H1N1) 2009 virus infection.

The entrance of the infection through the roadways is in agreement with the description by the patients concerning possible contacts with infected patients and is suggested

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Table 4 Multiple logistic regression

Dependent variables	Multiple logistic regression			Multiple logistic regression: optimized model		
	OR	*P*-value	95% CI for OR	OR	*P*-value	95% CI for OR
Age	0.983	.002	[0.972; 0.994]	0.982	.001	[0.971; 0.993]
Cough	4.177	< .001	[2.684; 6.500]	4.450	< .001	[3.082; 6.425]
Headache	1.482	.097	[0.931; 2.360]	1.451	.036	[1.024; 2.057]
Rhinorrhea	0.980	.929	[0.628; 1.529]	—	—	—
Dyspnea	0.421	.031	[0.192; 0.926]	0.418	.022	[0.198; 0.881]
Epistaxis	4.062	.043	[1.046; 15.777]	3.855	.044	[1.034; 14.372]
Sneezing	1.353	.263	[0.796; 2.300]	—	—	—
Fever	1.693	.070	[0.958; 2.992]	1.840	.019	[1.106; 3.060]
Myalgia	1.525	.103	[0.918; 2.536]	1.491	.042	[1.014; 2.194]
Vomiting	0.514	.014	[0.302; 0.875]	0.463	.001	[0.288; 0.744]
Diarrhea	0.706	.151	[0.439; 1.135]	—	—	—
Nausea	0.845	.602	[0.450; 1.588]	—	—	—
Number of symptoms	1.005	.9661	[0.791; 1.276]	—	—	—
Link*	1.384	.966	[0.967; 1.980]	—	—	—
P-value (model)	< .001	< .001				
Area under ROC curve	0.734	0.726				
Overall percentage	71.0%	71.7%				

95% CI indicates 95% confidence interval; OR, odds ratio; ROC, receiver operating characteristic.
*Link or epidemiologic link-existence of a stay or residence in a area where there was registry of transmission in the community of pandemic (H1N1) 2009 virus infection, during the 7 days prior to the beginning of symptoms.

by the greater incidence rates observed at the districts of Guarda and Coimbra.

In the central region of Portugal, young adults were the most affected, with a median age of 25 years. The distribution of the infection by categories according to the age showed that 89% of the patients were less than 29 years old having a specific incidence rate (35%) greater in the category of 20 to 29 years. The distribution observed in our region was similar to those reported in other countries and regions.[11,13,14]

The clinical aspects presented by the infected patients with the pandemic (H1N1) 2009 virus, showed that the majority of the patients (91%) presented fever or at least it was referenced, and this was the most common manifestation, being followed by cough, headache, odinophagia and myalgia. We also observed that these manifestations appeared in a similar proportion to the described in other European countries and the Unites States of America.[10,13-15]

We verified that diarrhea was described in a greater proportion of patients from occidental than from oriental countries.[11]

We found that about 9% of the patients were apyretic at the moment of the observation but had reference to fever prior to the clinical observation. The criteria for collecting samples for laboratory diagnosis were clearly defined on the working guidelines emanated from the Portuguese Heath Directorate (DGS), but in the last situation, the collection of samples was performed according to the clinical judgment of the attending physician.

Table 5 Precision of the definition of a *case for investigation* concerning the presence of fever at the moment of the clinical observation versus history of fever in the past days

	(H1N1) 2009			
	Negative (n = 573)		Positive (n = 255)	
Fever, n (%)				
Absent	94	16.4%	24	9.4%
Present	479	83.6%	231	90.6%
Total	573	100.0%	255	100.0%

Calculated Percentage by column.
Sensitivity: 90.6%; specificity: 16.4%.
Positive predictive value: 32.5%; negative predictive value: 79.7%.

This last aspect could be of public health importance, because the definition of a *case for investigation* adopted and previously described that justified the request for laboratory diagnosis, can exclude a significant proportion of cases of infection that have not formal indication to proceed for sample collection and laboratory diagnosis.

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The available literature on this subject shows that up to 33% of the cases of pandemic (H1N1) 2009 virus infection could have a clinical course with apyrexis.[11]

Clinical criteria of the case definition for investigation when analyzed on the perspective of the existence of confirmed fever at the moment of the clinical observation showed to be highly sensitive[8] allowing de identification of 91% of the cases of pandemic (H1N1) 2009 virus infection. On the other hand, as we observed in our study, the specificity of the definition was low (16.4%) but it is in agreement with previous case definitions for seasonal influenza.[16-18] The explanation comes from the possibility that some patients with history of fever in the last days but with absence of fever at the moment of the clinical observation, could have had no fever at all or then there is the possibility that some infections could have a course without fever or with very short periods of fever.

From the data presented in Table 4, we can observe that the positive predictive value (PPV) was 33% which means that the cases validated as *case for investigation* that presented fever at the moment of the clinical observation had a low probability of having a positive result in the diagnostic test for the pandemic (H1N1) 2009 virus infection.

The cases validated as *case for investigation* that at the moment of clinical observation do not presented fever but presented only history of fever during the last days, the probability to have no infection with the pandemic (H1N1) 2009 virus was high-predictive negative value (NPV) of 78%. These two last values are explained by the smaller proportion of pandemic (H1N1) 2009 virus infections (31%) observed in the context of the validated cases for investigation.

According to the data found in our study, the pandemic (H1N1) 2009 virus infection, was associated with a greater number of symptoms (5 symptoms versus 4, $P < .001$), and also some of the symptoms were predictors of a greater probability of infection (cough and epistaxis) than others. This aspect, suggest that the specificity of the definition of *case for investigation* for the pandemic (H1N1) 2009 virus infection, could be raised and will deserve a deeper analysis in later studies. We cannot leave without stressing that a raise in the specificity, based on data from our study, will be valid only for mild to moderate cases as we found.

From the confirmed cases of pandemic (H1N1) 2009 virus infection, we observed that in 97% of the patients fever, occurred at the same time or after other clinical manifestations have started. In only 35 cases, fever was the first clinical manifestation of disease. We did not find in the literature reference to the temporal profile of the appearance of the different manifestations in the epidemic setting.

Analysis of the available data concerning the early days of the pandemic allow us to declare that the severity of the cases observed varied from mild to moderate, similar to what was described in other countries.[10,19]

At this phase, there were no cases of pneumonia, respiratory failure requiring ventilator support or deaths, in the central region.

The present study shows some limitations. First, some patients infected with pandemic (H1N1) 2009 virus, with a clinical picture of a *case for investigation* that could justify a request for laboratory confirmation could not have looked for health care services or in the alternative, an unknown number of patients infected with the pandemic (H1N1) 2009 virus but with a clinical picture that did not fit the case definition could have never been submitted for laboratory confirmation.

The present study has also some strength that needs to be stressed, that is it included a sample of a well defined population embodying all age groups where the same laboratory diagnostic methods were used in a systematic way and where the clinical data was collected according to predefined standards and in agreement with working guidelines from the General Health Directorate.

Conclusions

The early days of pandemic (H1N1) 2009 virus infection were associated with cases of mild to moderate severity in our region and an explosive dissemination was not observed. Early detection and diagnosis combined with strict isolation procedures, as well as treatment and prophylaxis of the close contacts with oseltamivir could have contributed significantly to the slow spread of the infection in our region.

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Conflict of interest

Authors declare they don't have any conflict of interest.

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