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Historical Scope of Seasonal Influenza A and B Infections in Cuba and Their Impact on Mortality in the Elderly, 1983-2005

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Abstract: Background: Influenza causes high mortality in the elderly. Its behavior has not been studied regularly in tropical and subtropical countries, like Cuba. The purposes of this study were to understand the circulation type and subtype of the influenza virus and to obtain evidence of their relationship with underlying P&I deaths in persons aged 65 years or older. Methods: A retrospective study was carried out with 7,252 positive sera from influenza A(H3N2), A(H1N1) or from type B by hemagglutination inhibition assay from 1983-1984 and 2004-2005 seasons, which created three series with the type/subtype prevalence percentages corresponding to the total of positive cases. The relationship between mortality by pneumonia and influenza in people aged 65 years or more was determined by calculating its mean rates when the type/subtype prevalence was above the median or not. Results: The circulation pattern of these viruses in Cuba was defined, and predominance of A(H3N2) in the 77% of seasons was corroborated. The mean rate of mortality increased 24.4% ($p < 0.001$) when any pair of virus was above its medians and for the subtype A(H3N2) the increase was of 14.6% ($p < 0.05$). Conclusions: For the first time the Health Authorities of Cuba received a "native" pattern on the circulation of influenza viruses in the period studied, showing increases in mortality for underlying pneumonia and influenza in people aged 65 years or older when the A(H3N2) or any pair of viruses were above their means and underscoring the need for better prevention measures and vaccination programs for elderly people in our country.

Key words: Influenza virus, mortality, pneumonia, influenza, Cuba.

1. Introduction

Influenza viruses kill hundreds of thousands of people worldwide each year and cost society many billions of dollars in morbidity and lost productivity [1, 2].

Two influenza A virus, subtypes H3N2 and H1N1, and type B virus, have been circulating among human beings in the lasts decades. Epidemics caused by each of these types/subtypes vary from season to season. The major causes of these annual epidemics levels are a result of constant antigenic variation in an evolving influenza virus attempting to evade host protective immunity [3, 4].

The human society and health authorities have devoted great efforts to know and control influenza, organizing a global surveillance, led by the WHO with very high levels in the most developed countries, coinciding with the temperate zones of the planet and a lesser scope in tropical and subtropical ones creating important lack of knowledge on the epidemiology and the behavior of the virus.

In Cuba, a tropical and developing country, with 11 million of inhabitants approximately, located at 74 °and

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at 85 °W and 20 °and 23 °N [5], influenza seasons are not as well defined as in the template countries. Influenza virus in our island circulates all the year with a low or moderate activity and a biannual increase during September-October and January-April [6, 7]. Here, influenza virus surveillance is carried out through the year. Since 1974, clinical samples from patients diagnosed with acute respiratory infections (ARI) have been collected to determine the influenza types/subtypes circulating all over the country. These samples are processed at the National Influenza Center (NIC), located at the Influenza Virus Laboratory of the Pedro Kour í Tropical Medicine Institute. Complete epidemiologic information about ARI morbidity and mortality has also been compiled, based on this surveillance system [8, 9]. ARI is placed as the first cause of medical care within the infectious diseases while pneumonia & influenza (P&I) mortality take the fourth place among all causes of deaths, which is a very similar pattern to that from more developed countries [10]. P&I rate is an accurate indicator of influenza activity [11].

Classic and molecular diagnostic techniques have been implemented in the Cuban NIC [12]. Among the techniques used, since the beginning, is the hemagglutination inhibition assay micromethod [13], which determines the systematic circulation of A(H3N2), A(H1N1) and type B. In spite of counting on this broad information, this important disease has not been studied in Cuba at a long term. We have understood the elimination of this gap as a priority task. Our primary aim was to determine the characteristics of the annual circulation levels of both influenza virus and types/subtypes from the 1983-1984 to the 2004-2005 seasons in Cuba. The second aim was to obtain evidence of their impact on mortality.

2. Materials and Methods

We conducted a longitudinal and retrospective study of influenza virus types/subtypes circulation from the

1983-1984 season to the 2004-2005 season.

2.1 Season Definition

We define each annual influenza season as the period from September 1 through August 30 of the following year, taken this as our time unit, resulting in the 22 seasons included in the study [14, 15].

2.2 Virology Data

A total of paired serum positive samples were included (7,252), processed by (IH) [13] at the Cuban NIC within the time of study, and structured in three time series of subtypes A(H3N2), A(H1N1) and type B. IH was carried out with 18 reference strains of the subtype A(H3N2), 10 of A(H1N1) and 9 of type B. We considered a positive serum when there was a fourfold increase of antibody titers levels between pre- and post-infection sera.

The circulation of influenza viruses A(H3N2), A(H1N1) and B was determined for each season, dividing the positive sera of each virus by the total of positive ones. These percentages represent the presence of each type/subtype among the influenza-affected people within the population. This series of percentages were analyzed by calculating their averages, the standard deviation, the mean, the maximum and the minimum and their limits for a 95% confidence interval. Pearson's correlation coefficient was obtained to identify the relations among the three series, plotting them in a simple linear chart.

2.3 Expression of Changes of Circulation of Types/Subtypes on the Disease

We looked for serological evidence of types/subtypes variations impact on mortality, classifying each one of them by seasons when it was over or under its own median, and calculating the

pneumonia and influenza (P&I) mortality annual rates in people ≥ 65 years old per 100,000 people when each type/subtype was over its median and when it did not

reach this level.

Pneumonia and influenza mortality data were obtained from Cuban Vital Statistical Recorder, based on the International Classification of Diseases, ICD-9, codes 480-487 and 514 for 1979 to 1998 [16] and ICD-10, codes JO9-J18 from 2000 to 2005 [17] and the Cuban population from the national census and the projections of the National Office of Statistics (NOS) of the Republic of Cuba [18], estimated for June 30th.

The series of descriptive study was made with SPSS V 20, (IBM Corporation). The differences in the mortality P&I rates were determined by Student t test, calculating the equality of variances by the Leverene Test, of the same software, and were considered statically significant for $p < 0.05$ (95%, CI).

3. Results

3.1 Descriptive Studies

Within the 7,252 positive paired sera to influenza virus, 5,730 were type A, from which 4,128 were subtype A(H3N2), 1,602 subtype A(H1N1), and 1,522 type B. The three viruses were present in each season. Annually, the circulation of subtype A(H3N2) was predominant with a higher percentage ($\geq 50\%$) in 17 out of 22 seasons (77.3%), while A(H1N1) reached those values only in a season but type B did not (Table 1).

The mean of the yearly percentages of the seasonal circulation was higher for A(H3N2), average of 56.5% (95% CI, 50.9-62.2). Both the subtype A(H1N1) and Type B showed similar percentages, 21.9% (95% CI; 16.9-26.9) and 21.6% (95% CI; 17.5-25.7), respectively (Table 2). The three series had similar standard deviations: 12.8, 11.3 and 9.3%. The medians corresponded to 60.1% for A(H3N2), 21.4% in A(H1N1) and 21.6% for type B (Table 2). The coefficients of variation were higher in the subtype A(H1N1) and the type B with values of 51.7% and 42.9% and relatively low for the A(H3N2) subtype (22.7%).

The time series of A(H3N2) had its minimal level of

positivity (18.4%) in 2002-2003 and the maximal (83.2%) in 1987-1988, with a range of 64.8%. The A(H1N1) had extreme values contrary to A(H3N2) with a minimum of percentage (4.9%) in 1987-1988 and a maximum (52.7%) in 2002-2003 for a range of 47.7%. Type B had a minimum (6.0%) in 1986-1987 and a maximum (37.6%) in 1990-1991 with a range of 31.6% (Tables 1 and 2).

Pearson's correlation coefficients showed an inverse and high correlation of the subtype A(H1N1) with -0.712, regarding to A(H3N2), statistically significant (95% CI, $p < 0.0001$) and a lesser magnitude with the type B -0.51 (95% CI, $p < 0.015$). Between A(H1N1) and type B there was not significant correlation (Fig. 1).

The circulation of the types/subtypes showed another interesting characteristic related to the frequency of the local extremes, higher for the subtype A(H3N2) with 15 of these moments, whereas the A(H1N1) and the type B registered 11 and 9, respectively. This correlation in the behavior of seasons is shown in the simple linear chart of the positivity of types/subtypes (Fig. 1).

3.2 Effect of Types/Subtypes Changes over Mortality in These 22 Seasons

In these 22 seasons, the total of 93,711 deaths due to P&I mortality in ≥ 65 year old people, with a rate mean of 370.1/100,000 population showed a maximum of 476.6 in 2000-2001 and a minimum of 220.5 during 1988-1989 (Table 1).

The rate means differences, according to its associations with the A(H3N2), A(H1N1) and the type B level over and under their medians, were significant only with the A(H3N2) with a difference of 14.6% ($p < 0.05$). On the other hand, this comparison when any pair of types/subtypes, [A(H3N2) and A(H1N1); the A(H3N2) and type B or the A(H1N1) and the type B], were over its medians were higher and epidemiologically

Table 1 Influenza seasons, total of positive cases to A(H3N2), A(H1N1) and type B by seasons, percentages. Cuba, 1983-1984 through 2004-2005 season.

		Influenza virus						P&I mortality ≥ 65 years/100,000 people
		Influenza type A				Influenza type B		
		A(H3N2)		A(H1N1)		B		
Temp.*	Total of positives	Positives	%**	Positives	%**	Positives	%**	
1983-84	336	155	46.1	65	19.3	116	34.5	396.1
1984-85✝	505	346	68.5	46	9.1	113	22.4	436.7
1985-86✝	386	232	60.1	83	21.5	71	18.4	414.1
1986-87✝	319	192	60.2	108	33.9	19	6.0	398.8
1987-88✝	749	623	83.2	37	4.9	89	11.9	350.7
1988-89✝	613	316	51.5	197	32.1	100	16.3	220.5
1989-90✝	403	242	60.0	121	30.0	40	9.9	241.9
1990-91✝	298	163	54.7	23	7.7	112	37.6	293.8
1991-92✝	288	188	65.3	40	13.9	60	20.8	310.1
1992-93✝	442	268	60.6	94	21.3	80	18.1	380.2
1993-94✝	185	130	70.3	23	12.4	32	17.3	351.5
1994-95✝	249	132	53.0	32	12.9	85	34.1	334.0
1995-96	149	61	40.9	41	27.5	47	31.5	360.4
1996-97✝	135	71	52.6	30	22.2	34	25.2	409.3
1997-98✝	275	176	64.0	27	9.8	72	26.2	410.6
1998-99✝	314	193	61.5	40	12.7	81	25.8	400.9
1999-00✝	259	165	63.7	71	27.4	23	8.9	418.1
2000-01✝	89	55	61.8	28	31.5	6	6.7	476.6
2001-02	140	67	47.9	46	32.9	27	19.3	359.5
2002-03	653	120	18.4	344	52.7	189	28.9	371.9
2003-04✝	243	137	56.4	47	19.3	59	24.3	390.3
2004-05	222	96	43.2	59	26.6	67	30.2	416.0
Totales	7252	4128	56.5	1602	21.9	1522	21.6	370.1

Records/ National Influenza Center (NIC), IPK and National Estatistics Center, Public Health Ministry , Cuba.

✝ Season by influenza A(H3N2) virus (≥ 50%).

* Influenza season, from the month of September of a calendar year until the month of August of the following year.

** Percentages over the total of influenza viruses positives.

Table 2 Descriptive studies.

Influenza viruses series: A(H3N2), A(H1N1) and type B. Cuba, 1983-84 to 2004-2005

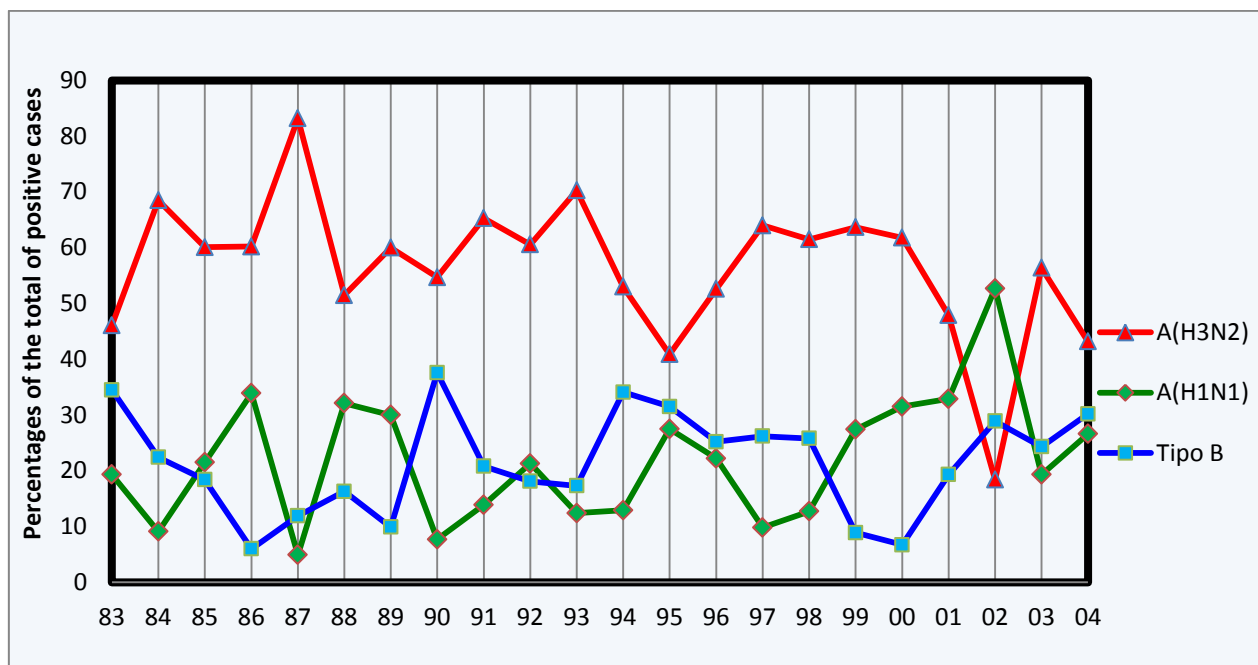| Seasons | | Influenza viruses types/subtypes | | |
		A(H3N2)	A(H1N1)	Type B
Means		56.5	21.9	21.6
Confidence intervals 95%	Min. Level	50.9	16.9	17.5
	Max. Level	62.2	26.9	25.7
Standard deviation		12.8	11.3	9.3
Coef. of variation		22.7	51.7	42.9
Median		60.1	21.4	21.6
Minimum		18.4	4.9	6.0
Maximum		83.2	52.7	37.6
Range		64.8	47.7	31.6

Records/National Influenza Center (NIC), IPK, Cuba.

Fig. 1 A(H3N2), A(H1N1) and Tipe B percentages of circulations by seasons, Cuba, from 1983-84 to 2004-05 Records/National Influenza Center (NIC), IPK, Cuba.

Table 3 P&I mortality means differences when each type/subtype where over its medians and when not, Cuba, 1983/84 to 2004/05.

		Influenza Viruses Types/Subtypes			
		One virus			Two virus over de median ‡
Mortality P&I ≥ 65 years		A(H3N2)	A(H1N1)	Type B	
Percentages	Season*	Mean rate	Mean rate	Mean rate	Mean rate
Over their median	11	395.30	371.55	383.64	410.31
Lower their median	11	344.88	368.63	356.55	329.87
Difference (%)		50.42 (14.62%)	2.92 (0.79%)	27.09 (7.60%)	80.44 (24.39%)
Stat. Signif. CI 95 %		0.05	0.91	0.311	0.001

Records/National Influenza Center (NIC), IPK and National Estatistics Center, Cuban Ministry of Public Health, Cuba.

* Influenza season, from the month of September of a calendar year until the month of August of the following year.

‡ Influenza season when a pair of virus—A(H3N2) with A(H1N1) or type B and A(H1N1) with type B—get percentage over theirs medians.

important, with a difference among the mortalities means of 80.44 (24.4%) and $p < 0.001$ for a 95% CI (Table 3).

The research limitations were found in the retrospective approach, shown mainly in the impossibility to incorporate the evidence on the circulation of other respiratory viruses and the variations in the amount of samples processed by season, demanding to focus the study on the time series of the frequency of each type and subtype respect to the total of sick people with influenza diagnosed in the laboratory and not with the population, as a whole.

4. Discussion

This is the first report in Cuba on the activity of types/subtypes of influenza virus during an extensive lapse. According to our purpose, this retrospective vision has contributed to reducing our influenza virus circulation knowledge gap in Cuba, a tropical country.

We highlight the increases in the P&I mortality in people with ages ≥65 years, taking the influenza year as time unit, when any pair of viruses coincided with the circulation levels over its respective medians and, with a lower level, when this evaluation was made, taking

seasons with A(H3N2) over these levels, without taking into account the others virus levels. For us, these results demonstrate the predominant impact on P&I mortality of subtype A(H3N2), with its systematic and high presence, without denying or presupposing a not despicable action of the A(H1N1) subtype and type B by their own variations, leading to the increase in the P&I mortality in people aged ≥ 65 years.

The descriptive analysis showed similar consistent patterns of circulation and behavior, to those reported for these viruses worldwide [19], emphasizing the presence of the three influenza virus in all seasons with means and inter-seasonal variation with potential to impact on the epidemiology of the disease.

Among the three viruses the A(H3N2) stood out by its high mean and by the number of seasons in which it reaches incidence levels higher than 50.0% (17 seasons), which is representative of the high levels of exposition of the Cuban population to this subtype, which in comparison with the A(H1N1) and the type B, according to their means, determined a risk 2.6 times higher to get sick with the influenza virus A(H3N2) than the A(H1N1) or the type B ($p < 0.0001$) in Cuba.

Previously-published reports in Cuba agree with the fact that A(H3N2) is the predominant subtype with the highest circulation levels of the types/subtypes, although they refer a reduced number of seasons and reduced geographical spaces [20, 21]. Aponte et al. [22], during the 1987-1988 season, exploring the circulation of these viruses in the Eastern region of Cuba, confirmed the predominance of A(H3N2) followed by the subtype A(H1N1) and the type B with 52.7, 29.4 and 17.8%, respectively. Reports from CDC of Atlanta highlight similar results in this season in China, Singapore, France, Finland, Italy, Spain and Canada [23].

Influenza surveillance in the world accounts for the recurrent epidemics and recognizes the predominance of subtype A(H3N2) in most seasons, followed by a minor number of cases by the subtype A(H1N1) and type B [24-27].

Finkelman et al. [28], analyzing the global patterns of the seasonal activity of influenza viruses at a wide geographical scale of temperate countries with data confirmed of the subtypes A(H3N2), A(H1N1) and type B during 1997-2005, showed in 19 countries an average prevalence between 47 to 73% for the subtype A(H3N2). Countries with similar percentages were Japan (50.9%), France (59.7%), Romania (59.8%) and Denmark (59.5%).

Influenza A(H1N1) had similar levels to those of the present study in Argentina (22.3%), Japan (20.6%), Spain (20.8%), Germany (22.4%), although the general mean of all the countries included in this paper was lower (14.6%). In the type B there are coincidental values in South Africa (23.6%), Austria (22.8%), Japan (28.7%), Portugal (24.5%), Italy (21.6%), Romania (25.4%), England (23.6%) and Lithuania (21.9%) [28].

The results of this paper are consistent with reports from those countries, where the three influenza viruses were present in a simultaneous way in the 82% of the seasons, with a higher coincidence in tropical areas of Singapore [29], Taiwan [30], and the South of China [31], where the presence of A(H3N2), A(H1N1) and B was detected in 92% of the seasons.

The standard deviation variability with respect to the means of the seasons for the different types/subtypes (Table 2) is another important characteristic described in this report, which could be interpreted as expression of the antigenic-immunity cycle change, because of the strong impact of this disease on the population, given the ability of these viruses to cause infections in the host, reflected in the P&I mortality, mainly in the elderly, and co-morbidity with a chronic disease [32, 33]. The literature reviewed also shows high variation rates in countries like Taiwan [30], the South of China [31], the United States [32], with significant differences among them. As in Cuba, these countries report the A(H1N1) with the highest figures.

Cuban reports show a better correspondence with Singapore [29], a tropical country, where the A(H3N2) and type B variability were 26.3% and 48.3%,

respectively. The subtype A(H1N1) provided the highest rate of variation in both countries.

The negative Pearson's coefficients of correlation between the A(H3N2) and the A(H1N1) suggest a dynamic influence between both subtypes, indicator of their recognized competitive interference during the sequence epidemics, related to an existing heterosubtypic immunity that reduces the probability of reinfections [34].

The proposed dynamic and the lack of correlation of the type B with both A subtypes and the differences in the frequencies of the local extremes in the simple linear chart of these viruses show the different rhythms of the antigenic changes and the predominance of one or another type/subtype. However, little is known about the genomic scale evolutionary dynamics of the pathogen and its relation with these changes [4, 34-36].

Studies performed in different countries based on data of the excess of deceased associated to seasonal influenza from 1980 to 2004 in Portugal [37] are associated to a rate of 24.7/100,000 people and were 3-6 folds higher during seasons dominated by the A(H3N2) subtype and in Canada influenza virus accounted for 13.0 (1990 to 1999) [38]. In China, in five cities of the South with subtropical climate 11.3/100,000 people [31] and in the US an excess of 19.6/100,000 people was estimated [32].

In Europe, the excess of mortality rates was valued with varying values between 16/100,000 in Germany during the period 1985-2001 [39] and 26/100,000 during influenza epidemic in the Czech Republic through 1982-2000 [40]. In Netherlands it was estimated for a 22.5-year period (1967-1989) in which, as average, more than 2,000 people died from influenza each year, in the same populations [41].

5. Conclusion

Our initial goals were achieved. Here we have a picture of the influenza virus circulation patterns in Cuba, which state its differences and similarity with other countries and regions, which increase our capability of orientation in front of this threatening disease. This has also confirmed the importance of viral circulation data to support not only historical investigations but the operational control as well.

We should also highlight our finding of the virus circulation association with the disease and its high impact on mortality, when comparing these variables between two groups of seasons, in correspondence with the virus prevalence circulation levels. We understand this is an evidence of the virus circulation decisive role as causal factor of high mortality, given its expression through the elevated number of causal factors that should be acting in a really wide temporal horizon used in this work.

This research also contributes to understanding influenza in tropical countries, mainly in the Caribbean islands. The results support the yearly anti-flu vaccination to Cuban population of people aged 65 or more and underscore the need to improve measure control.

Conflict of Interest

The authors deny any conflict of interest associated with the publication.

Acknowledgments

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