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Start of a Pandemic: Influenza A H1N1 Virus

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1. Introduction

In Mexico, Influenza virus infections occur more frequently during the winter with small peaks throughout the year. In April 2009, an epidemic arose from the emergence of a new Influenza A virus, whose devastating effect reflected significantly in Mexico city. Numerous patients with Influenza-like severe symptoms were attended at the Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas, Mexico (INER) and in many cases required hospitalization. The INER, in collaboration with the Dirección General de Epidemiología (Mexico) and the Instituto Nacional de Referencia Epidemiológica, Mexico (INDRE) marked the first guidelines for the management, diagnosis and treatment of Influenza-illness in Mexico. The INER is considered one of the leading centers for the care of critically respiratory-ill patients, and was one of the first institutions to describe the clinical presentation and death in the initial period of outbreak (Acuña, 2010; Manjarrez et al., 2003). The pandemic that occurred in 2009 in Mexico, took a different tack in contrast to other countries with respect to the frequency, severity and number hospitalized patients with mortality and greater affection in healthy young people. Influenza is a respiratory illness caused by viruses of the same name. Each year, seasonal flu viruses originate occasional epidemics. The name "influenza" comes from the belief that disease was caused by a negative supernatural influence, that is, a malign influence, or that the stars have influence on the occurrence of the disease (Acuña, 2010; Manjarrez & Arenas 1999).

In Mexico, there exist accurate records of 13 severe epidemics occurred during: 1667, 1734, 1805, 1806, 1826, 1837, 1846, 1891, 1899, 1918, 1920, 1957 and 1969, being reported the highest rates of mortality in the year of 1919 (Acuña, 2010). Infections are most common during the winter and small outbreaks over the year (Manjarrez et al., 2003, 2010).

2. Characteristics of the influenza virus

2.1 Classification and nomenclature

Influenza viruses are grouped in the Orthomyxoviridae family including three types: A, B and C. The A gender is the most common, infecting birds and mammals, including humans;

it can causes epidemics and pandemics with high rates of morbidity and mortality. Influenza viruses B and C are less frequent, mainly infecting humans and causing moderate epidemics or outbreaks (Manjarrez & Arenas 1999; Manjarrez et al., 2010; Souza, 2011; Zambon, 1999). According to the antigenic characteristics of hemagglutinin (HA) and neuraminidase (NA) proteins, influenza viruses are subdivided into subtypes. To date, it has been characterized sixteen HA subtypes and nine NA antigenic subtypes, all of which are found in aquatic wild birds. The international nomenclature used to describe the different influenza virus is to appoint the following characteristics: a) gender of the virus A, B or C; b) host type is ignored when it comes from human c) place of isolation; d) case number in the laboratory; e) year of isolation; f) HA and NA subtype of the virus which is written in parentheses. An example of a human virus isolated in Mexico in 2009 was described as "A / Mexico / INDRE / 4487 / 2009 (H1N1)" (Dawood et al., 2009; López-Cervantes et al., 2009; WHO, 2009a).

2.2 Structure

Viruses range in size from 80 to 120 nm in diameter having a pleomorphic or spherical form. They have a segmented genome (7 and 8 fragments) of single stranded RNA of negative polarity, encoding 11 proteins being two glycoproteins. Fragments 1, 2 and 3 encode proteins PB1, PB2 and PA those are part of the polymerase complex and are involved in the synthesis of RNA. Segment 4 encodes the HA protein which is the receptor that binds to the cell. It is synthesized as a monomer which undergoes posttranslational modifications and is activated by a host protease that cuts it into two fragments, HA1 and HA2, exposing the peptide fusion in the aminoterminal region of the HA2 fragment, allowing fusion of the viral envelope with the endosome in order to release the genome into the cytoplasm. The mature protein is a trimer formed by a stem that is inserted into the membrane and three globular regions. The globular region has an amino terminal region, where are localized the antigenic sites that are surrounding the receptor binding site (Manjarrez & Arenas, 1999; Zambon, 1999). The fragment 5 encodes the nucleoprotein (NP), forms a complex with RNA, protects the genome and is involved in replication. Segment 6 encodes for the neuraminidase (NA), the mature protein consists of a stalk and a globular head constituted of 4 subunits each with a catalytic site. Its role is to remove sialic acid residues of the membrane of the infected cell, allowing the newly synthesized virions were not clumping together and released properly. Segment 7 corresponds to the M1 and M2 proteins that are synthesized by a overlaid reading frame. M1 protein surrounds the nucleocapsid and M2 is located in the membrane and functions as an ion channel allowing the transport of protons to the endosome and is the target for antiviral drugs (e.g. amantadine). Segment 8 encodes for two nonstructural proteins: NS1 and NS2, that is, not belonging to the virion; these proteins are synthesized when the cell is infected. The NS1 protein inhibits the production of interferon; however, the function of NS2 protein is still unknown. Recently, it has been described the PB-1-F2 protein with a pro-apoptotic function (Manjarrez et al., 2010; Zambon 2001; WHO, 2002). Influenza viruses have a high rate of molecular variation, changes that can cause epidemics and pandemics. These changes are given by different factors: the nature of the virus (RNA replication needs of a RNA polymerase, which is inefficient to correct errors, so that mutations are common, in addition, the genome is segmented allowing increased recombination. Influenza A viruses infect humans as well as a variety of animals, this allows high levels of recombination between viruses from two or more hosts. Among

the known mechanisms of variation there is the "antigenic drift", which are small changes that lead to mutations, mainly in the two viral glycoproteins (HA and NA). This variation allows an ineffective function of the host neutralizing antibodies, contributing to the need of renew vaccines annually. Other variation mechanism that is less frequent is the "antigenic shift". This mechanism allows the generation of new subtypes that can arise by recombination and rearrangement of entire segments of genes (Figure 1).

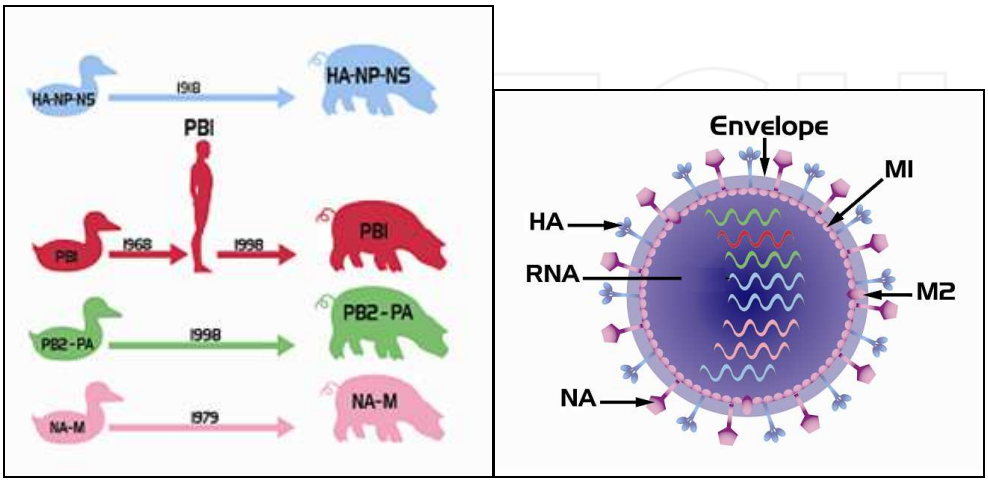

Fig. 1. Pandemic Influenza Virus. The new virus has a reassortant, the changes are shown in different colors: PB2, Classical North American Swine H1N1/Triple reassortant swine H3N2/A avian-like; PB1, Classical North American Swine H1N1/Triple reassortant swine H3N2/A avian-like; PA, Triple reassortant swine H3N2/A avian like; HA, Classical North American Swine H1N1; NP, Classical North American Swine H1N1/Triple reassortant swine H3N2; NA, Eurasian Swine N1; M Eurasian Swine; NS, Classical North American Swine H1N1/Triple reassortant swine H3N2.

2.3 Viral transmission and pathogenesis

The virus enters through the nasopharyngeal region for aerosols and droplets expelled to talking, coughing and sneezing, with hands and objects contaminated with aerosols and can last for hours or days on contaminated surfaces. The virus infects mucus-secreting epithelial cells and ciliates cells.

The HA binds to cell receptor, after incubation for one to three days, the virus replicates rapidly and infects neighboring cells. The virus causes cell damage, which alters the cilia activity, with increased secretion of mucus. To get out and infect other cells the NA reduces the viscosity of the mucus.

Desquamation on the epithelium causes respiratory symptoms and signs, involving the respiratory tract's natural response and promotes bacterial incorporation. Other damage is on the submucosal edema, which in turn may lead to hyaline membrane disease, emphysema and alveolar necrosis. The inflammatory process may break bronchi, bronchioles and alveolar regions. All these events originate characteristic initial symptoms

of infection such as: fever, chills, and widespread pain, particularly muscle aches, headache, anorexia and prostration. The local monocytes and lymphocytes are the primary response and interferon is effective against the virus. The virus induces an effective humoral response, it is important in recovery, but should be considered that the antibody response is specific for each variant of the virus, while the T lymphocytes and macrophages is general and depending on the damage and the condition of host, epithelial repair can take up to a month. Start the virus infects upper respiratory tract but, it can reach low way causing bronchitis, bronchiolitis and pneumonia.

3. A new pandemic caused by a new influenza A virus in 2009

Usually in Mexico, Influenza infections occur in winter (Cabello et al., 2006; Vandale et al., 1997); however, in March 2009, began to be evident the first signs of influenza infections, an unusual time for the viral infection. The spread was fast and it was extended to many countries. On April 30th, the World Health Organization called officially an "H1N1/09 Pandemic Virus" (Dawood et al., 2009; Garten et al., 2009; López-Cervantes et al., 2009; Updated 2009; Vaillant et al., 2009; WHO, 2009). So, on June 11th, the WHO declared a pandemic. Mexico City showed the most serious cases with numerous deaths, the causal agent was found to be a new type of influenza virus. To June 15th, in 76 countries the virus was detected with 35,928 cases of infection and 163 deaths. By July 17th the virus had spread to 135 countries, with 101,250 confirmed cases. In Mexico, July 17th, Mexican Ministry of Health reported that the virus was present in all 32 states of the country; the number of cases was 13,646 with 125 deaths. Of the 70.4% recorded deaths was found in the age group of 20 to 54 years, the deaths accounted for 0.9% of total deaths, of which 52% were women, most of whom were housewives (Secretaria de Salud de Mexico, 2010b)

When the pandemic virus appears, there may be several periods of outbreaks with a range of 3 to 9 months between them. The pandemic in Mexico occurred in three outbreaks. The first began with the epidemiological alert on April 17th, and lasted for about a month. The second was in the months of June-July in the Southeast, although the magnitude was much smaller than the first outbreak. The third began in September 2009, mainly in northern and central Mexico.

On November 27th, the WHO informed that more than 207 countries had reported the presence of the new virus (Vaillant et al., 2009; WHO 2009). Here is a brief narrative of the activities, measures and experiences that took place in the INER at the onset of the influenza pandemia.

4. Approach of influenza medical cases at the respiratory emergency unit

It was received a disproportionate demand of patients showing severe acute respiratory infection (SARI) in addition to other possible influenza patients with other pathologies. Therefore, the mechanisms of classification, prioritization and selection of hospital management were reinforced. For the epidemic emerged in Mexico were implemented three phases of Triage:

1. **Initial Triage.** Patients with potentially SARI were separated from those with other diseases. Patients were required to fill an application form assessed by a physician.

2. **Second Triage**. Patients with respiratory symptoms related to influenza A H1N1 infection or potential epidemic/pandemic infection were removed from patients with infection by other viruses or bacteria.
3. **Third Triage**. It was defined the location of patient: home, hospital ward or intensive care.

Triage is useful for infection control and epidemiological monitoring, as well as the rationalization and organization of the Respiratory Emergency Unit overcoming potential responsiveness.

Classification systems include schemes to determine the severity of community-acquired pneumonia and influenza; this classification is not necessary when the risk groups are different so they could be misclassified probably conducing to an increased mortality in young patients during the pandemic. The proposed schemes should only be considered as a guide and do not replace the clinical criteria (Corrales, 2010; WHO, 2007). Among the schemes implemented are: Criteria for classification of patients; calculation Score: Pneumonia Severity Index for Adult (PSI/PORT), which consider the characteristics of patient demographic factors, comorbidity disease, findings on physical examination, laboratory and/or radiographic findings (ranked by points) (Corrales 2010, Osterholm 2006); Risk Rating PORT severity index of pneumonia, and Scoring System CURB-65 (See next box).

SCORING SYSTEM CYRB-65	
FEATURES	**POINTS**
Confusion	+1
Urea> 7mmol/L (20 mg/dL)	+1
Respiratory rate > 30 breaths per min.	+1
Blood pressure (systolic > 90 or diastolic < 60 mm Hg)	+1
Age > 65	+1

Patient Score	**Site Recommended care**
0-1	outpatient
2	Ward
3-5	Hospital room or intensitive care unit

Table 1. Criteria for classification of patients

Unfortunately, all efforts to use these scales in viral pneumonia, especially those caused by human influenza virus A H1N1 during the 2009 pandemic have been unsuccessful and unreliable.

5. Considerations for selection of patients on critical care medicine

It was considered two components of Triage: 1) prioritize patients and 2) management and resource optimization. This system is not error-free (overtriage or undertriage).

Experts in disaster situations have established a classification system or Triage at the Emergency Department or Intensive Care Unit. This system consists in to establish a color system for assessment and patient selection: green (patient will survive or not receiving support), yellow (patient will survive in case of delayed medical care); red (patient needs immediate intervention to survive), blue or black (patient will die despite the best efforts of Physicians).

However, the situation that we live during the first outbreak of pandemic influenza experienced at the INER during March to May was truly different and represented a challenge for the treatment of many patients with severe respiratory failure requiring mechanical ventilation or in shock.

Unable to attend all patients because the Department of Critical Medicine has only 9 intensive care beds (2 of them for pediatric patients) and 5 intermediate care beds, it was decided to transform the cubicles of pediatric patients into care beds for adults. This action allowed us to had 15 beds.

Moreover, the conversion took place in hospital besides enabling a pavilion, also allowing the use of this area for the care of patients with influenza A H1N1 pneumonia with or without need for mechanical ventilation in this situation; we had to suspend the no outpatient urgent and elective surgery activities in order to concentrate human resources in these areas. According to these conditions, patients most likely to survive (e.g. under 65 years old without comorbidity in end-stage cancer, without multiple organ failure and/or without terminal renal failure with requirement of replacement therapy because there was no equipment for (renal replacement T-Hemofiltration and hemodyalisis). They were accepted preferably in the Department of Critical Medicine, that was our initial triage system. We organized a committee Influenza Hospital (Bautista, 2010; Funk et al., 2010). Patients who were not chosen for their attention to a critical care area, represented a difficult situation because although they should be given immediate attention, establish a prognosis according to their severity and set neatly in the family may even be conflicting even among physicians in different areas within the hospital, but in a pandemic is only a problem in addition to a long list that puts the resource limit with which it has in every way, physical area, technology, inputs and humans. To decide which patients should be hospitalized and then locate them in the level of care they should receive may be more complex (outpatient observation in the emergency room, hospitalization, need for critical care or in spite of the latter by requiring extremely serious and not being able to triage proportions) creates a serious problem.

Regarding to resources, it was necessary to make a list indicating the available material and which must have, number of mechanical ventilators, beds, protective equipment for personnel, drugs and so on. It should be performed together involving CEOs, medical and administrative personnel, nursing, Medical Committee etc., for monitoring and troubleshooting. Several aspects of the strategy included area marking, media provide reports to the press and interested staff, health staff compare with that account and assign roles and responsibilities, flexibility of spaces, since in an emergency usually mechanical ventilators, beds in the different units and services which are not enough to make adaptations (Sandoval, 2010).

This indicates that the triage is necessary for the success of a medical contingency. Triage has medical basis for their development. It is necessary that each hospital unit set their standards according to their structure and needs (Sandoval, 2010; Rubinson et al., 2008).

6. Characteristics of the clinical cases

Clinical cases of influenza-like illness showed respiratory insufficiency and later was confirmed the infection with influenza A (H1N1) virus. Patients were mostly young individuals, in less than one year had the highest rates, with a gradual decrease in inverse proportion to the age (Figure 2). However, it was found that complications occurred in 10% of adults who were hospitalized. With increase age after 35 years, it was shown the highest percentage (20%); people over 65 years-old required hospitalization. Same behavior was observed with regard to lethality, with an increase on 20 to 35 years-old group as well as in the age group over 65 years-old. Because of the severity of respiratory damage, a large number of patients required intubation (mean age: 37 years-old). Fifty percent of all cases were female with no significant differences in mortality and hospitalization. Co-morbidities were frequent and varied in patients: the most common were diabetes mellitus, hypertension, chronic obstructive pulmonary disease, obesity, smoking and pregnancy (Bojorquez et al., 2010; Behazin et al., 2009; Cabello et al, 2012 in press). Patients who presented with a severe respiratory illness were immediately isolated.

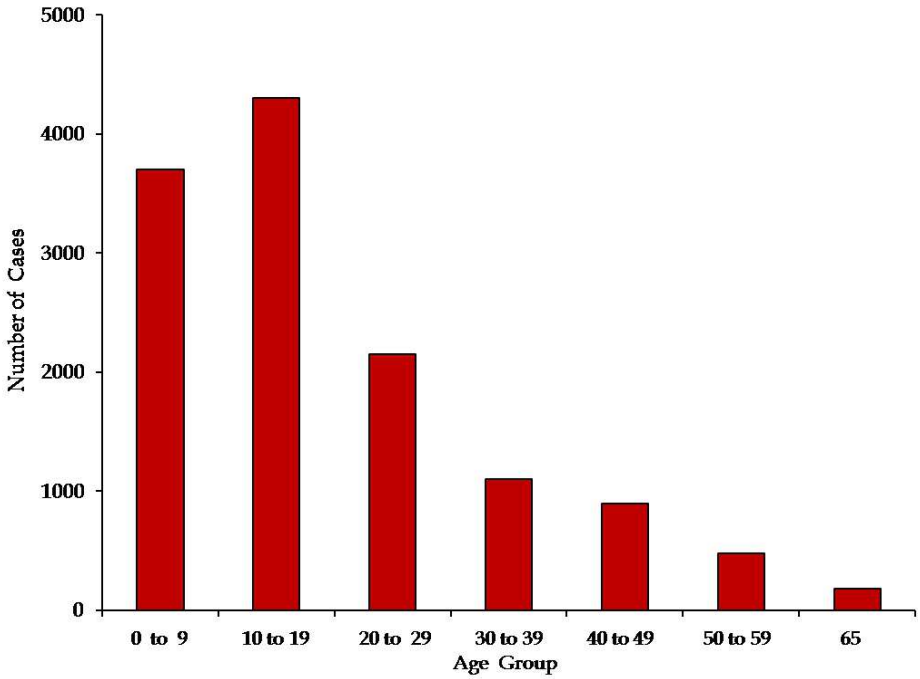

Fig. 2. Influenza cases in Mexico by age group from 11th March to 27th May, 2009. Data obtained from: Fajardo, et al. 2009.

7. Clinical manifestations

In general, the clinical manifestations observed during the infection tend to be similar with any subtype of influenza virus. The characteristics of the disease included signs and symptoms ranging from mild to severe manifestations. In Mexico, at the beginning of the pandemic of 2009, it was introduced a delay in diagnosis because the patients first consulted a general practitioner who were given treatment with antibiotics. Once identified the circulation of a new influenza virus, most patients (over 50%) had self-limiting illness without medical care. Complicated cases arrived at a hospital were treated with antiviral treatment, considering that if 72 hours after was not an improvement; illness were managed in accordance with the recommendations for progressive disease treatment. In mild cases, damage was mainly in upper respiratory tract, and the main symptom was dry cough. When the infection spreads to the lower airways, bronchus and lung parenchyma, it causes pneumonia with cyanosis and sometimes bloody sputum. Signs and symptoms were as follows:

Cough. Cough occurred between 90 and 100% of cases. Initially, illness was unproductive and then, in 33% of patients showed bloody sputum.

Dyspnea. It was presented in 65-73% of patients with complicated disease, so it was considered as the main symptom indicating the severity of the disease evolution. This symptom was presented in 86% of patients with pneumonia and in 90% of patients requiring management in the Intensive Care Unit (Cabello et al, 2012 in press; Pérez-Padilla et al., 2009; Echeverría-Zuno et al., 2009; Domínguez-Cherit et al., 2009). Sore throat and runny nose was observed in patients with upper respiratory tract infection (75% of patients) and in patients requiring hospitalization (35%) (Pérez-Padilla et al., 2009).

Sore throat and rhinorrhea. It was noted that patients with upper respiratory tract infection occurred in 75 and in patients requiring hospitalization, about 35% had (Pérez-Padilla, 2009).

Fever. Temperatures over 38°C were considered as the main systemic symptom increasing with the severity; in mild cases occurred in 40-50% while in severe cases occurred up to 100% (Cabello et al, 2012 in press; Pérez-Padilla et al., 2009; Echeverría-Zuno et al., 2009).

Headache. It was more common at the onset of the disease and was more frequent in outpatients than inpatients. We recorded 22 to 88% of the cases (Cabello et al, 2012 in press; Pérez-Padilla et al., 2009; Echeverría-Zuno et al., 2009; Domínguez-Cherit et al., 2009).

Myalgia and arthralgia. It was reported mainly in muscles of the lower extremities and occurred between 40 to 70% of total of the cases.

Gastrointestinal symptoms. Usually, seasonal influenza has a frequency of 5% while influenza caused by the new virus was reported between 10 to 30% of cases and was more common in children. It has been suggested, although not proven a fecal-oral transmission (Petrosillo et al., 2009; Ramírez et al., 2010).

Diarrhea, nausea and vomit. In seasonal influenza are rare, but when the disease is more severe it has been reported in 3 to 5% of the patients. At the INER the new virus was reported in 10% of severe cases in comparison with other medical centers in which it was reported a positive infection in 20 to 38% of cases.

At the beginning of the outbreak, there was a transmissibility rate of 1.2% and a mortality rate of 0.4%. Subsequently it was reported new ranges of mortality, from 0.20 to 1.23%, of which the lower ranks were in Europe and the highest in Mexico. It has been reported in Mexico during the pandemic that in Mexico city was the highest number of deaths in comparison with other countries, and the increased risk of death from pneumonia included people over 60 years of age. At the INER from April 17th to December 4th, 2009, it was reported a total of 763 cases of pneumonia with 138 deaths accrued. There have been some possible explanations for this situation: being the first country in which began the outbreak, the virus had an increased pathogenic activity and spread, therefore, patients to be unaware that this was a new virus, failed to get adequate medical care. In addition, medical staff was not aware of the existence of new viruses conducing to mismanagement of patients. In children, the clinical picture of the new virus influenza A (H1N1) infection was launched as seasonal flu-like infection, fever greater than 37.5°C, cough, chills, headache, nausea, hyperoxia, lethargy, muscle pain and fatigue, but added gastrointestinal symptoms such as diarrhea and vomiting. The clinical spectrum of symptoms was mild to progressive respiratory distress syndrome, requiring mechanical ventilation, associated with organ failure and death. Finally, the main causes of death were respiratory failure, sepsis, dehydration and electrolyte imbalance (Cabello et al, 2012 in press; Cano et al., 2010; Fajardo et al., 2009; Garrido et al., 2010).

8. Histopathological features of the disease detected in patients admitted to the INER

8.1 Tracheobronchial abnormalities

The seasonal influenza virus A and B have an affinity for epithelial cells and produce ciliated epithelial erosion and ulceration and inflammatory infiltrate in the subepithelial layer, initially neutrophils and later by lymphocytes. Macroscopic autopsy confirmed patients infected with influenza A (H1N1) virus showing extensive hemorrhagic tracheobronchitis, ciliated columnar epithelium with extensive necrosis, intraluminal desquamation of epithelial cells with partially eroded basement membrane, few epithelial cells with cytoplasmic vacuolization and deposition of hyaline membranes focally. In patients who died within hours after hospital admission showed no inflammatory cells. Patients with longer history had few neutrophils in the *lamina propia* of the mucosa while in patients with longer time of hospitalization, it was observed numerous lymphocytes infiltrating the tracheobronchial wall. Other findings observed were: vascular congestion and interstitial edema. In past pandemics caused by influenza virus infection, these observations have been described by other authors (Taubenberger & Moreno, 2008; Jeannette et al., 2006).

8.2 Pulmonary histopathological findings

While the new virus caused several deaths, macroscopic lung findings were not very different from those described in seasonal influenza. The *post mortem* biopsy specimens and autopsy cases showed massive pleural effusion predominantly on the right, lung weight and larger size, shiny outer surface, heterogeneous areas of congestion and hemorrhage of bright pink and red violet color; these lungs were poorly demarcated, confluent, giving a

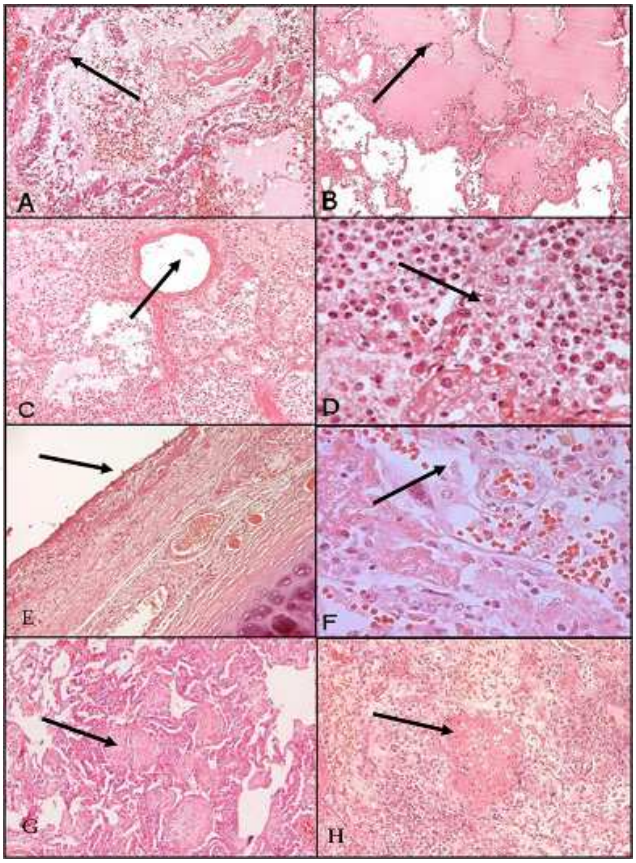

Fig. 3. **Micrographs of damage in the lung parenchyma and tracheal:** A) Respiratory bronchiole epithelial ulceration, fibrin plugs and scaling of columnar cells and macrophages; B) Alveolar edema and hemorrhage; C) Necrosis of the alveoli with deposits of hyaline membranes and alveolar edema with heavy infiltration by neutrophils and congestion of the capillaries; D) Interstitial edema with infiltration by neutrophils, capillary dilation and intraalveolar edema; E) Wall of the trachea is observed denudation of the respiratory mucosa; F) Hyperplasia of type II pneumocytes and cytomegaly; G) Areas of bronchiolitis obliterans due to proliferation of fibroblasts; H) necrotizing vasculitis surrounded by acute inflammatory infiltrate. Hematoxilyn and eosin stain (HE).

marbling pink to red-violet color, soft consistency and some consolidated areas; some cases showed lobar consolidation (Vázquez et al., 2010). Microscopically, most cases showed diffuse alveolar damage in exudative phase, with marked alveolar epithelial necrosis and hyaline membrane formation up to 5 microns thick, with focal dilatation of alveolar spaces, intra-alveolar hemorrhage, edema, hyperplasia and scaling of Type II pneumocytes and macrophages in the alveolar pinocytosis. In some macrophages and pneumocytes cytopathic damage was observed characterized by nucleomegaly, thrombosis with appearance of frosted glass capillary and necrosis of alveolar septa. It was identified areas of congestion

and capillary alveolar wall thickening. According to the evolution time and complications, leukocyte infiltration between predominantly neutrophilic and lymphocytic lineage was little more intense in areas with few megakaryocytic intracapillary necrosis. Thrombosis of the capillary rise of the alveolar wall necrosis in large areas, confluent with fibrin exudate and loss of alveolar epithelium adjacent to the intra-alveolar hemorrhage and diffuse alveolar damage of acute lung inflammation was observed as well as occasionally peribronchial and bronchiolitis with necrosis and ulceration of the epithelium, which corresponds to the typical histological pattern of viral-induced lung damage. There were signs of tissue repair, regeneration of the bronchiolar and alveolar epithelial hyperplasia, and squamous metaplasia of pneumocytes (Vázquez et al., 2010). In the interstitium and bronchioles (bronchiolitis obliterans) was observed proliferation and reorganization of fibroblasts (Masson bodies) as well as large areas of fibrosis. Other authors (Guarner et al., 2000) detected influenza virus infection in bronchial epithelium and submucosal glands of the trachea, bronchi and bronchioles. Figure three shows some of the histopathological findings.

8.3 Findings using electron microscopy

By examining several sections of the lung, the ultrastructural changes that were detected were: alveolar capillaries with numerous erythrocytes and some leukocytes, airspaces with Type II pneumocytes, with projections in discrete cytoplasmic membrane and occasionally adjacent debris. In the cytoplasm of some macrophages phagocytosed erythrocytes and autophagosomes (electron concentric deposits) were observed. Type II pneumocytes have abundant cytoplasm with mitochondria-like swollen smooth endoplasmic reticulum which tend to lose their crests; in addition, round nuclei with little heterochromatin attached to the inner nuclear envelope and a prominent nucleolus were found. Low viral particle number, ranging from 93 to 150 nm in diameter, was visualized in the cytoplasm of some alveolar epithelial cells. At different stages of the viral cycle, it was observed vacuoles and endosomes emerging from the cytoplasmic membrane of the pneumocyte. Alveolar epithelial cells showed moderately dilated smooth endoplasmic reticulum, presence of electron-slightly larger vacuoles (compatible with pulmonary surfactant), round to oval nuclei with little heterochromatin in the nucleoplasm and prominent/eccentric nucleoli (Vázquez et al., 2010).

9. Imaging studies

The first choice study was simple plate-ray or chest X-ray; the techniques used were the portable X-ray and teleradiographs. The most frequent pattern was mixed alveolar and interstitial, predominantly axial and bilateral basal respecting the periphery. Cases with rapid progression were observed following the consolidation respecting upper lobes without associated lymphadenopathy or pleural effusion. In severe cases, the progression was rapid and affection pattern reverted to 18 to 30 days. Some patients underwent high-resolution computed tomography. Reconstructions were completed after 1 second, reconstruction algorithm and the high end of a forced inspiration, also took a series on forced expiration from lung apex to the diaphragmatic dome. The pattern found was mixed (interstitial and alveolar), predominantly interstitial type, characterized by areas of unpolished glass with a trend of consolidation, predominantly basal bilateral, axial

compliance lung apices sometimes patchy pattern. Associated effusion or lymphadenopathy were not found. Expiratory phase showed areas of air trapping (Abella, 2009; Alva & Perdigón 2010; Chowell et al., 2009).

Figure four shows some of the findings in radiographic images.

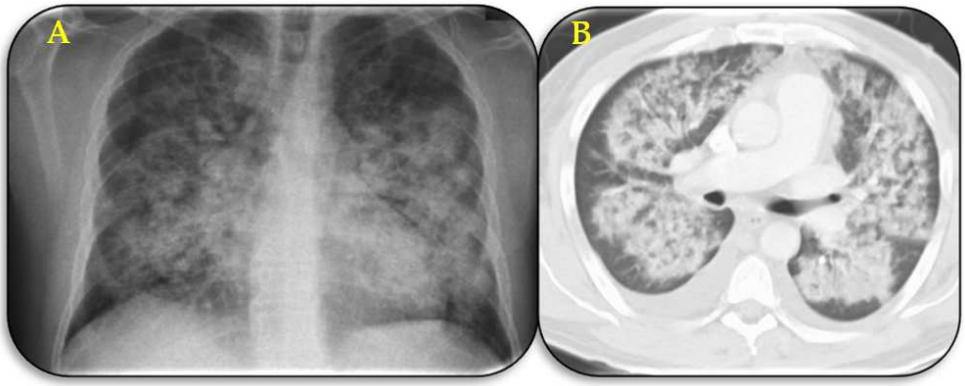

Fig. 4. Male patient 47 years of age diagnosed with H1N1 and 15 days earlier. A) The radiograph shows bilateral alveolar opacities; B) TAC Atypical pneumonia, and diffuse alveolar damage.

10. Respiratory therapy strategies for the management of patients with influenza A H1N1 virus

One of the important points in the respiratory therapy strategy involves knowing all the devices used in patients with some degree of respiratory failure and that includes everything from ways to deliver oxygen, nasal cannula, masks in all its varieties combining with moisture systems and the need to administer bronchodilators and/or inflammatory airway. In patients infected with the influenza A H1N1 virus, the use of bronchodilators and inhaled anti-inflammatory drugs is required. It is recommended the use of thermal units with systems of automatic filling and thermal self-regulation, reducing the handling of samples and the risk of dispersion of viral particles into the environment. Is important to avoid the use of thermal units that promote water condensation in the ventilator circuit. This situation requires the frequent disconnection of the circuit promoting environmental contamination with viral particles. Filters/nose or HME filter (Humidity Moisture Exchange) generate passive moisture and prevent the passage of bacteria into the patient allowing the safely change of filters every 7 days (Garcia & Garcia, 2010; Thorpe & Darcy 2009). We recommend active humidity systems for mechanically ventilated patients with high PEEP and are candidates for the use of bronchodilators. If the patient does not require the use of bronchodilators or other medications in nebulizer, nose/filter is a good choice. In the case of circuits of mechanical ventilation, the new generation ventilators compensate for leaks in the circuit. These systems can be cleaned safely each 7 days, even 15 days, in both cases, as long as the circuit does not show contaminating secretions. The interaction between the circuit and thermal units, and misting systems or aerosol delivery are important and we

recommend a combination in the setting to the minimum necessary to disconnect or unplug it. Disconnection is suggested for each clamp on the tracheal cannula after the inspiratory phase, decreasing the risk of viral contamination into the environment. The processes of oxygenation in patients with influenza include devices such as nasal prongs, masks (opened and closed; high and low flow). All these systems have shown that in the process of exhaling, sneezing and coughing are generated particles including viral particles. The oxygen delivery systems in patients with influenza and respiratory failure depend on the severity of respiratory symptoms, the greater hypoxemia, and more oxygen is required. The administration by nasal cannula provides an inspired oxygen fraction up to 40% in order to maintain or improve oxygen saturation (oxygen partial pressure above 60 mmHg) (Garcia & Garcia, 2010; Tellier, 2009; Jefferson et al., 2009).

It still necessary studies to optimize the management of patients with influenza and to generate techniques to minimize risks to medical staff, nurses, technicians and anyone who interacts with the patient.

11. Extrapulmonary complications in pneumonia due to influenza A H1N1

Pneumonia caused by Influenza Virus A (H1N1) corresponds to Acute Respiratory Distress Syndrome (ARDS) with increased intra-alveolar hemorrhage. In ARDS, the increased mortality due to no respiratory complications is common in the critically ill patient; 16% of patients may develop a complication that includes: sepsis and septic shock, myocarditis and pericarditis, acute encephalitis, rabdomielitis, acute renal failure, liver failure and/or disseminated intravascular coagulation. In patients with chronic obstructive pulmonary disease or asthma, viral infections are frequent causes of complications.

12. Respiratory failure and multi-organ in Influenza-induced illness

In the first cases of influenza in Mexico, that included 18 patients, people required hospitalization, oxygen therapy and required mechanical ventilation due to ARDS. Among the reported data it can be included: elevated body mass index elevated LDH or CPK as well as history of smoking. Epidemiological data of patients with respiratory failure, who required intensive care, have been described in two series of patients in Mexico City and Canada (Echeverría-Zumo, 2010; Kumar et al., 2009). In both series the age groups are similar (40-50 years), showing, respect to seniors, a peak in early childhood and a significant variation in mortality for hospitalized patients (48 and 14% respectively) and oxygenation index [ratio of arterial oxygen pressure to fraction of inspired oxygen (PaO 2 / FoO2)], founding significant differences in the need for early mechanical ventilation (less than 10 hours), renal failure, hypotension at admission or shock refractory to intravenous fluids or need for vasoactive drugs and elevated values of lactic dehydrogenase, although the latter difference was not significant. Other interesting facts included decreased arterial oxygen saturation in patients who required mechanical ventilation (average 71%) and severe degree of hypoxemia in the group of nonsurvivors (41.5 mmHg) (Bautista et al., 2010; Dominguez-Cherit et al., 2009; Chavarría et al., 2010). For the proper management of patients with any acute or chronic lung disease exacerbated is important to integrate all data and knowledge.

13. Diagnosis

The speed with which the diagnosis is made is directly related to virological techniques employed. Techniques can be used to detect viral antigens using immunofluorescence or ELISA (Enzyme-linked immunosorbent assay) assays, which allows having a fast and reliable diagnosis. Novel detection technique such as Influenza A (H1N1)-real-time PCR (AH1N1-RTqPCR) was recommended recently by the WHO (CDC, 2009a, 2009b; Poon et al, 2009; Public Health 2009). This assay allows discriminating between different strains of seasonal and pandemic influenza. This technique uses specific primers to amplify specifically fragments of genes that codify the influenza virus Hemagglutinin or M2 proteins. The RTqPCR can detect up to 5,000 copies of a gene in each sample, and it can be used combined set of primers for determining different viral strains in a single biological sample. During the A (H1N1) pandemic, this technique was implemented as a main part of a diagnostic platform allowing the detection of new viral mutations of the recent A (H1N1) pandemic strain (Public Health, 2009; Pabbaraju et al., 2009; Balish et al., 2009). For the determination of neutralizing antibodies, the WHO suggested hemagglutination inhibition test (CDC, 2009).

The techniques used for early diagnosis of influenza directly dependent on the type, quality and quantity of biological sample and for the detection of viruses. On the recommendation of the WHO, we used different types of samples such as bronchoalveolar lavage samples, aspirates, nasal and nasopharyngeal swabs or aspirates (Balish et al., 2009; Ison, 2009; Thorpe & Darcy 2009). For patients who are intubated, it was considered the recollection of endotracheal aspirates (Balish et al., 2009; Thorpe & Darcy 2009).

In order to minimize the possible spread of disease, during the sampling was used appropriate work equipment. For the transport of biological samples was important to consider the use of specific culture media for viruses as well as the use of equipment or refrigerants to maintain a temperature of 4°C, which help to reduce the loss of virus collected.

14. Therapeutic management

Usually, the majority of flu cases showed mild and minor symptoms or manifestations, are self-limited not requiring antiviral treatment instead only symptomatic measures such as analgesics, antipyretics like acetaminophen or ibuprofen. During the influenza pandemic were considered for treatment the following clinical features: persistent fever, progressive symptoms, risk factors such as pregnancy, obesity, infants with alarm data. When not requiring antiviral therapy were given directions to go back to see if they have alarm data such as dyspnea, fever difficult to control, bloody sputum or changes in consciousness. In case of children, they were monitored to the presence of changes such as cyanosis, tachypnea and apnea. Before treatment and for the proper management of patients were considered some aspects: Treatment should be initiated as soon as influenza is suspected since when management delayed for more than 48 hours is not very effective, in severe cases should be initiated as soon as possible, even if have been more than 48 hours. In cases of pneumonia, it has been described that treatment was effective in patients who have symptoms for more than 6 days, helping to decrease mortality. Treatment will probably not prevent the development of ARDS, or cytokine storm, but prevents serious illness and

death. Another important aspect is that treatment can reduce the need for hospitalization and length of hospital stay; in addition, there exists the need to know if comorbidity or data that might indicate a progressive evolution (Cabello et al, 2012 in press; CDC, 2009; Higuera et al., 2011; Sada, 2010; WHO, 2009b).

14.1 Antiviral drugs

The dose of oseltamivir administered to adult patients was 75 mg, every 12 hours for 5 days. In cases of pneumonia, it should use double dose, i.e. 150 mg every twelve hours, being able to prolong treatment for ten days. The adult dose of zanamivir was two doses of 10 mg by inhalation every twelve hours for 5 days having the disadvantage of producing irritation of the airway that could lead to bronchospasm, so caution should be exercised with patients with prior respiratory problems, such as asthma and chronic obstructive pulmonary disease. In some cases is recommended the prophylactic administration of bronchodilators prior to the application of zanamivir. In the case of intravenous neuraminidase inhibitor, peramivir, the recommended dose is 600 mg every 24 hours. Pediatric doses of oseltamivir should be calculated by age and weight, according to the following recommendations: 30 mg twice daily if ≤15 kg; 45 mg twice daily if weight is 15 to 23 kg; 60 mg twice a day for weight from 23 to 40 kg; 75 mg twice daily if weight > 40 kg. In children under 5 years is not recommended the use of zanamivir while in children over 5 years old, doses are similar to those of adults: two inhalations of 10 mg every twelve hours (CDC, 2010; Hanshaoworakui et al., 2009;Kidd et al., 2009; Sada, 2010; Updated, 2009; WHO 2009b).

During pregnancy, it has been shown that antiviral drugs are effective; however, it should be administered early to prevent the development of pneumonia; fortunately these drugs have not been associated with congenital malformations.

14.2 Antimicrobial treatment

In severe disease there may be some bacterial respiratory complications in these cases it can use the treatment guidelines for community-acquired pneumonia. In the schemes recommended by Mexican experts, it has decided that all patients with pneumonia should also receive antiviral drugs such as ceftriaxone at a dose of 2 g intravenous or intramuscular every 24 hours for 7 to 10 days. Patients who require mechanical ventilation are not exempt from acquiring a nosocomial infection, so antibiotics were used according results of bacteriological analyses (Sada, 2010; Updated, 2009a, 2009b). In specific cases of patients with influenza, in addition to specific antiviral treatment is recommended other symptomatic treatments such as antipyretics and anti-inflammatory drugs (paracetamol or ibuprofen) at useful doses. It was considered contraindicated the use of aspirin, especially in children and young people and their use in influenza is associated with development of Reye's syndrome.

15. Pre-pandemic phase

In Mexico, in 2003 it was created a multidisciplinary group to develop a National Plan for preparedness and response to pandemic avian influenza, which was completed in 2006 (Manjarrez, 2007; Secretaría de Salud de Mexico, 2010 a). For the situation that arose in 2009, this Plan was resumed (Figure 5) in accordance with the recommendations of the WHO, the

Ministry of Health participated through the National Epidemiological Surveillance and Disease Control (CENAVECE), the Department of Epidemiology (DGEPI), the Coordinating Committee of the National Institutes of Health and High Specialty Hospitals (CCIN Salud HAE) in collaboration with entities of the health sector and government sector. In this document is showed local actions for a possible pandemic of avian influenza, in order to reduce cases of human infection and mortality. Main action lines of action for strategic activities were: 1) Dissemination and information, 2) Epidemiological surveillance, 3) Confirmation of the diagnosis, 4) Care for the population, 5) Strategic reserve and 6) Research and development.

Fig. 5. National Plan for preparedness and response to pandemic influenza in Mexico.

15.1 Pandemic phase

The National Plan for preparedness and response to pandemic influenza in Mexico was not fully consolidated, requiring dissemination of guidelines and operational training as well as antiviral supplies in all health institutions in the country, including non-governmental health institutions. When detonated the alert in Mexico, it began negotiations for the preparation, packaging and distribution of oseltamivir to all healthcare facilities across the country, which reported suspected or confirmed cases of A (H1N1), which caused a delay in the timely administration of the first patients affected by the pandemic (Manjarrez, 2010; Secretaría de Salud de Mexico, 2010).

Among the most outstanding activities was the diagnosis as a crucial part of epidemiological surveillance to monitor the A (H1N1) virus circulation correlating with the

occurrence of severe pneumonia cases. It could be proposed that this action was transformed to a vulnerable point of the health system and a point of discussion in the committees, because main resources and efforts were limited only to patients with pneumonia.

Hospital authorities adopted, as strategy, reference mechanisms since the facilities were inadequate, especially intermediate and intensive care units where many cases required mechanical ventilation; in addition, care activities from specialist staff was insufficient for local needs. Influenza committee sessions were performed daily in order to solve the main problems: the shortage of human and material resources. Other problems were: limited coverage of seasonal influenza vaccination, access to health services, availability of information on health, socioeconomic level, demographic characteristics, cultural background, educational level, government policies on health, etc. (Secretaría de Salud de Mexico, 2010). Mexico was the first country that gave international epidemiological alert in March and April 2009. According to official reports of surveillance were involved several states including San Luis Potosi, Hidalgo, Queretaro and Mexico City (Central Mexico). The Secretary of Health of Mexico reported the viral infection on April 24th (Secretaría de Salud de Mexico, 2010), therefore, social distancing measures were intensified to contain the pandemic, which included the closure of certain public places like restaurants, schools, kindergartens, cinemas, theaters, foot-ball stadiums, among others, these actions were extended until May 11th to Mexico City and May 18th for the rest of the states. In contrast to the global alert issued by WHO, Mexican pandemic rose to five on a scale of 6 on April 29th (fig.6).

15.2 Post pandemic phase

After the pandemic, there were disseminated, through various information media, criticism of the decisions taken by the authorities of the Ministry of Health in Mexico, because these radical actions to contain the dissemination of viral infection, National economy were crashed. However, this strategy played an important role in advancing on the field of epidemiology and public health that included:

- Improved hand hygiene in the population.
- Control of antimicrobials through medical prescription.
- Activation of epidemiological surveillance systems.
- Research resources.
- Investment in health infrastructure, including medical equipment such as mechanical ventilators.

It was developed several guidelines and rules to follow, including:

15.3 Precautions to prevent transmission in hospitals and health care

- Preventive isolation of patients, including a combination of standard and transmission precautions
  - Direct contact
  - Drop spread
  - Air spread
- According to the demands, ideally patients should stay in a room with negative pressure, or to use a single room whose door will remain closed most the time. When

institutions do not have these resources by the magnitude of the pandemic, patients should stay in multiple rooms or rooms specially designated for patients infected with influenza. The litters must be a minimum distance of 1 m.

- Health professionals, directly involved in patient care must use high efficiency masks as N-95 or its equivalent, long sleeved gowns with adjustable cuffs, face shield or goggles and gloves. This equipment is not recommended for administrative professionals that do not require direct contact with these patients as is recommended face masks only.
- It is necessary to limit the number of health professionals in direct contact with the patients and to limit the access to the patient environment.
- Health professionals who attend patients with pandemic influenza should not take care of patients diagnosed with other disease or young children.
- Restrict visits to a minimum.
- Give visitors the regulation of hospital safety and personal protective equipment with instructions for use.
- Encourage frequent hand washing, particularly in areas of risk by the presence of influenza patients.

15.4 Security measures at an unprotected exposure of health professionals or family members

1. Professionals who care for infected patients should monitor their temperature twice daily and report any febrile episode. If exist any sign or symptoms, the professional should not to participate in non-specific or direct care of patients.
2. Health professionals who have a fever (temperature > 38°C) and have been in contact with patients should undergo appropriate diagnostic tests. If no other cause is identified, it should be assumed an influenza infection and immediately start treatment with oseltamivir.
3. Healthcare professionals involved in high-risk procedures (e.g. procedures which generate aerosols) that begin with any signs or symptoms should consider the need for prophylaxis.

15.5 Precautions for the general population during an influenza contingency

1. Household contacts should take appropriate steps in terms of hygiene; not sharing utensils, avoid face to face contact with patients with confirmed or presumptive diagnosis and the use of high-efficiency masks and googles (e.g. N-95 type).
2. Contacts who have shared a particular environment: home, homes of other family members, hospitals, schools, workplace, recreation centers, mass transport, etc., with a patient with confirmed or suspected influenza A (H1N1) should monitor their temperature twice daily as well as the appearance of symptoms within 7 days after the last exposure.
3. Confirmed asymptomatic contacts should not receive oseltamivir.
4. Symptomatic household contacts with temperature > 38°C, cough, dyspnea, diarrhea or other systemic symptoms, should receive empirical antiviral therapy (e.g. a dose of 75 mg once daily for 5 days in adults and children) and undergo to diagnostic testing as soon as possible.

15.6 Preventive measures for travelers going to areas with Influenza alert

1. People who are traveling to areas with avian influenza activity should be immunized with trivalent human vaccine available (seasonal), two weeks before the trip.
2. Travelers should avoid direct contact with poultry, including chickens, ducks, or geese that appear healthy, in addition, these people should avoid farms or live animal markets, poultry, or touching surfaces contaminated with feces of poultry secretions.
3. Travelers should take preventive measures to reduce risks of exposure through proper hygiene and frequent hand washing with alcohol-gel. Also, avoid eating eggs or foods derived from poultry that haven poorly cooked or raw.
4. Hand washing is important when handling raw poultry for cooking, either at home or in business.
5. It is necessary to recommend to travelers to consult a physician if the traveler has fever and respiratory symptoms during the 10 days after of returning from an affected area.

15.7 End of the pandemic

Health Secretary lifts the state of epidemic alert on June 30th, 2010. The latest official data, published by the Ministry of Health on June 19th, 2010, indicated that Mexico recorded 72,548 confirmed cases and 1,316 confirmed deaths, representing a mortality of 1.8%. Seventy percent of deaths corresponded to the age group between 20 and 54 years old, and 39.8% of them did not find any pathological history relevant to death (Secretaría de Salud de México, 2010a, 2010b).

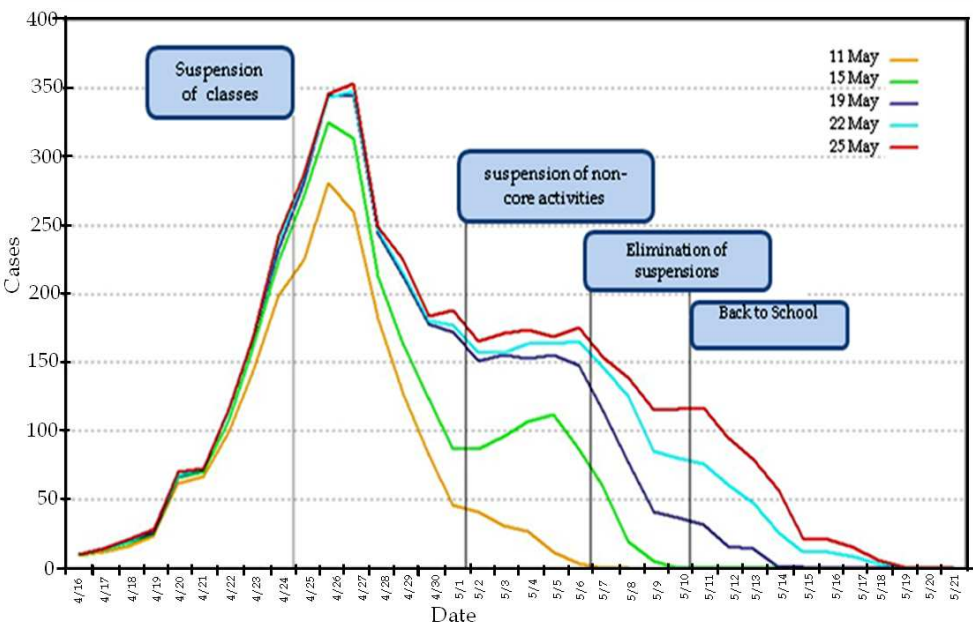

Fig. 6. Confirmed cases according to the date of symptoms on the population

16. Social response to the epidemic

The first manifestations of the epidemic in Mexico caused significant morbidity and mortality in the population, with implications for high psychological, social and economic impact (Cruz & Corrales 2010). With the emergence of a new influenza virus, the growth of reports of confirmed cases of illness and deaths caused the mass media fix their attention on it, the news spread very fast because it had little knowledge of the virus, generated a great concern throughout the population, disrupting social stability that had already been altered for several reasons.

The social response to an epidemic is given in circumstances that occur in time, space, era and culture, and serve to properly interpret phenomena according to social reality. An important aspect to understand this response is the psychosocial vulnerability, an internal condition of a subject or a group of subjects exposed to threatening or traumatic event, which corresponds to the perception of risk in which the person has to be damaged. Perception can be objective (real threat) or subjective (built by the subject, it may be consistent with reality or the risk can be distorted and exaggerated or minimized). Since the many kinds of risk perception, the situation led to a series of emotions and behaviors appropriate or inappropriate to the society. Adequate collective behaviors refer to protection measures that will allow reducing the spread of the virus and thereby reduce the number of cases, eliminating to fall into chaos. Inappropriate behaviors such as spreading rumors that encourage mass hysteria or that the situation is unreal will generate maladaptive behaviors that gradually lead to social disorganization (Cruz & Corrales 2010).

One of the feelings experienced by people was the collective fear, which is characterized by sharing an intense fear in a social group. This fear can be adaptive, allowing people to grade their level of risk and therefore take steps to protect themselves. As example, masks, antibacterial, vitamin supplements such as vitamin C and subsequently antiviral drugs were sold out. The collective fear can lead to exaggerated and disproportionate behavior of reality; one example: the population stopped eating pork since fear of contagion, as the initiation of pandemic was related to the swine flu.

Fear can turn into panic, manifested by an intense fear that can produce primitive reactions, messy and can take led to violence and discrimination (Cruz & Corrales 2010). Mexicans, who were abroad or want to leave the country, were badly treated and their individual rights breached. Some countries set restrictions on commercial and business flights that were suspended. Discrimination against Mexico and its people was very severe.

The coordinated effort of health workers, authorities, officials and civilians were able to maintain adequate adjustment mechanisms, promoting the restructuring of social welfare and allowing the development of active strategies for protecting the population and reduce the spread of the virus and ultimately, drive the epidemic successfully.

17. Detection of humoral immune response in different populations

The new influenza virus circulated and remained stable in the environment, easily entering the body. The immune system is activated to produce antibodies, which in any way prevent or reduce the development of the disease. For information from the presence of specific antibodies against the new influenza virus, we performed a multicenter study, which were obtained several results mentioned here only a brief summary of some of them.

1. Anti-virus influenza A (H1N1) pandemic in people at different levels of exposure to the virus, whose aim was to: Identify and compare the presence of specific serum Ab against the new virus influenza A (H1N1) and GEA INER staff at different levels of exposure. This study concluded that greater exposure, higher titers of antibodies and the application of the specific vaccine enhanced the response.
2. Prevalence of influenza virus A (H1N1) in blood donors. In this study analyzed 241 samples of sera from donors, by the method of hemagglutination inhibition (HI) for antibodies against influenza virus seasonal and pandemic virus. Samples were taken from November 2008 to May 2009. We found that in 65 (27%) were detected antibodies to the novel influenza virus and seasonal influenza virus 87 (36%). Found that only 26 (10%) samples were positive for both viruses. The titles ranged from 1:10 to 1:80. By comparing the results of the pre-pandemic period the epidemic found a statistically significant difference [OR 6.1,95% (2.34-14.1) p <0.0001]. Concluded that there are few studies showing the prevalence of the pandemic virus in a healthy population without recent illness. Although the results are few, indicate that the population was in contact with the new virus probably since 2008 the end of 2008 antibodies were detected and the prevalence in this population ranged from 9% pre-epidemic period and 36% epidemic period.
3. Another objective was to compare the presence of antibodies against the new influenza virus and seasonal influenza viruses INER staff and a Technology University of Tecamac (UT). This study concluded that the greatest number of people with antibodies to both viruses was INER, being significant in the case of the new virus, indicating that their exposure was greater when dealing with infected patients. It is interesting to note that INER staff is annually vaccinated against the virus, but number of people with seasonal virus antibodies was low.

The presence of antibodies may provide guidance on some aspects such as proximity or degree of exposure to the virus the ability to respond immunologically to viral stimulation, virus circulation:the possibility of partial or weak protection of a vaccine.

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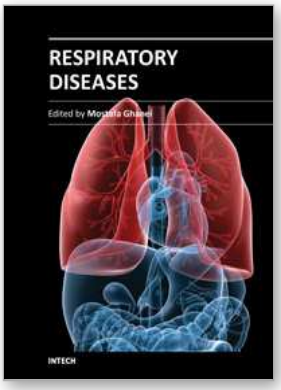

Respiratory Diseases
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Medicine is an ever-changing science. In this regard, Respiratory medicine is not an exception and has been evolving during recent years. As new research broadens our knowledge, advanced methods for diagnoses are better understood, providing genetic and underlying pathophysiology of diseases and new clinical experiences. Consequently, publications of new resources along with revisions of previous ones are required. The book Respiratory Diseases brings practical aspects of pulmonary diseases. It contains the result of years of experience through expert clinicians in this field from different scientific centers. The respiratory diseases are discussed according to epidemiology, pathology, diagnosis, treatment, and prognosis. It includes updated resources of the pathogenesis and some molecular aspects of the aforementioned diseases and is recommended reading for all clinicians and medical students, especially pulmonologists, to access highlighted respiratory diseases in this book.

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