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Review

The 1918 Spanish influenza: integrating history and biology

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ABSTRACT – In 1918 an influenza pandemic killed 40 million people. It is now possible to study the genetic features of the 1918 virus. Such analyses will try to answer questions about the origin and the unusual virulence of this pandemic virus. © 2001 Éditions scientifiques et médicales Elsevier SAS

Spanish influenza / influenza A virus / neuraminidase / hemagglutinin glycoproteins / history of medicine

Savoir pour prévoir.
—Auguste Comte (1798–1857)

1. Introduction

In the autumn of 1918, as thousands of soldiers were dying in the trenches of France, thousands more were dying in barracks and hospitals of what would come to be known as the 'Spanish flu'. While the war largely confined its ravages to the armies of Europe and the United States, the Spanish flu showed no such restraint, killing men, women and children all over the world. Almost 9 million people were killed over the 4-year course of the war. Between 20 and 40 million people were killed by influenza in just 8 months [1]. The virus causing the influenza pandemic was not isolated at the time. By the characteristic symptoms and epidemiology of the disease, contemporary observers identified the disease with influenza outbreaks of the past. However, its enhanced severity, its multiple waves within just one year, and its predilection for the young and healthy all suggested that this influenza outbreak was unique.

Since 1918, a great deal has been learned about influenza [2]. The first human influenza viruses were isolated and cultured in 1933 [2–4]. Gradual changes in the surface proteins of the virus were found to be responsible for the yearly recurrence of influenza epidemics [5]. Acquisition of antigenically novel surface proteins was discovered to be responsible for the pandemics of 1957 and 1968 [6–8]. The natural reservoir of influenza viruses was identified as wild aquatic birds, from whose populations viruses with new surface proteins could emerge through reassortment [9, 10]. However, it is still not possible to predict how and when new pandemic influenza strains will emerge, nor how virulent new strains will prove. Study of the 1918 strain allows us to add to the collection of pandemic strains [11–13], hopefully elucidating the mechanisms of reassortment and host adaptation. Furthermore, as the most deadly influenza virus ever experienced, the 1918 strain offers a unique potential to understand the connection between genotype and virulence.

2. History of the pandemic

There were two major waves of influenza in 1918 [14]. The first began in March of 1918 and spread unevenly through the United States and extensively in Europe over the next 6 months. Morbidity was high, but mortality was not appreciably above normal. Were it not for the explosion of the second wave in September, it is likely that the spring wave would have passed unnoticed. However, contemporary observers noted similarities between the two waves that led them to believe that, despite the marked difference in mortality, they were observing the same disease [15, 16]. Clinically, the illnesses were identical, although cases were much milder in the first wave. However, the rapid progression to fatal pneumonia that became well-known in the fall wave was already noted in the relatively few severe spring cases. As noted by LeCount [16], who conducted autopsies during both the spring and fall waves, "We did not know at that time (in April 1918) what we had. The lungs were full of hemorrhages…It was not until the fall that we knew what we had, when we had cases duplicated." This statement suggests that the spring and fall wave strains were substantially similar. The ability of the virus to replicate deep in the lung was already present in a very small minority of cases.

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Epidemiologically, the first and second waves were also similar [14]. The disease spread rapidly through a community, with incidence peaking after a few weeks and then rapidly declining. Morbidity ranged from 20–50%. A wave-like spread across geographic areas was noted. However, the spring wave did not become truly pandemic. While it was very extensive in Europe, and there are reports of outbreaks in US military camps and sporadic outbreaks elsewhere, the spring wave did not diffuse as widely as that of the fall. The first European outbreaks of the spring wave occurred in March in France, spread to England in June and did not reach Scandinavia until August. By contrast, the fall wave spread so explosively that the peaks of morbidity and mortality were reached throughout the world during October and November of 1918.

This description of the first wave suggests that by the spring of 1918, an influenza strain with a novel hemagglutinin surface protein had already emerged. It was adapted to replication in humans and was capable of human to human transmission. Since it was antigenically novel (judging from the very high morbidity rates), it spread extensively. However, the relative mildness of the illness and the length of time it took to spread suggest that it was not yet as perfectly adapted to humans as it would later become.

It is possible that the spring wave did not represent the emergence of the pandemic virus, but instead was a drift epidemic caused by a mutation in the hemagglutinin of the previously circulating influenza virus. However, the historical record suggests otherwise. In those places that experienced both the spring and the fall wave, almost all observers agreed that victims of the first wave either escaped or experienced only mild illness in the fall [14, 17–19]. Military doctors noted that troops that had been in a camp during a spring outbreak had much lower rates of infection in the fall than troops that had arrived in the summer [17]. Similar experiences were noted in institutions [14]. These reports indicate that the spring and fall wave viruses probably shared substantially similar hemagglutinin genes.

The potential of the virus changed in late August. Over the course of September and October, fresh waves of influenza spread from ports and urban areas throughout every continent. The speed with which the disease became entrenched was explosive. In one army camp in the United States, hospital admissions rose from an average of around 80 per day through the 9th of September to over 1 000 per day in the middle of the month [14]. Generally, the peak of local outbreaks was reached by the third week. Such rapid spread indicates that each victim infected many contacts, in other words, that the virus was highly transmissible.

Transmissibility is affected by many factors, such as host adaptation, antigenic novelty and dose. It is also affected by how well the different influenza genes work together. The processes of infection, replication and release from host cells require efficient cooperation among the virus' genes and between viral and host proteins. All of these factors affect the severity of illness produced. The pathology of autumn wave cases is consistent with a virus that replicated to extremely high levels, quickly infecting epithelial cells throughout the respiratory tree. The ability of the virus to cause damage deep in the lungs, in cells lining the alveoli, was unusually prevalent in 1918. Such extensive spread may reflect an ability to replicate to exceptionally high levels or a change in the type of cells the virus was able to infect. The combination of rapid spread and severe illness suggests that the influenza strain that emerged in the fall of 1918 was both antigenically novel and extremely well-adapted to replication in humans.

If one were able to compare the complete genomes of the spring and fall strains, one would predict a mutation or group of mutations that substantially improved the virus' fitness in humans. For example, the hemagglutinin protein might have an improved ability to bind to receptors on human cells or the neuraminidase an improved ability to release budding viruses. Replication and host adaptation are complex, polygenic processes. It seems likely that over the course of the spring wave the virus accumulated mutations in several genes or, possibly, acquired better adapted genes by reassortment, that made it significantly more efficient.

Like other pandemics, the 1918 influenza spread in waves. However, the rapid succession of two distinct waves that differed markedly in virulence is one of the factors making the 1918 pandemic unusual. In many places, there was yet another severe wave of influenza in early 1919 [14]. Three extensive outbreaks of influenza within 1 year is unusual, and may point to unique features of the 1918 virus that could be revealed in its sequence. Interpandemic influenza outbreaks generally occur in a single annual wave in the late winter. The severity of annual outbreaks is affected by antigenic drift, with an antigenically modified strain emerging every 2–3 years. Pandemic influenza is often not confined to a single, winter outbreak but rather diffuses throughout the world over the course of many months. In 1957, for example, the pandemic virus emerged in Asia in February and spread westward in sporadic outbreaks throughout the spring and summer, culminating in major outbreaks in the fall of 1957 and winter of 1958. The 1890 pandemic began in the late spring of 1889 and took several months to spread throughout the world, peaking in northern Europe and the United States late in 1889 or early 1890. The second wave peaked in spring 1891 (over a year after the first wave) and the third wave in early 1892 [14]. As in most pandemics, subsequent waves seemed to produce more severe illness so that the peak mortality was reached in the third wave of the pandemic. The three waves, however, were spread over more than 3 years, in contrast to less than 1 year in 1918. It is unclear what gave the 1918 virus this unusual ability to generate repeated waves of illness. Perhaps the surface proteins of the virus drifted more rapidly than other influenza strains, or perhaps the virus had an unusually effective mechanism for evading the human immune system.

Another distinctive feature of the 1918 pandemic, which might also be related to the interaction of the virus with the human immune system, is the unique age incidence of mortality from the 1918 virus. The 1918 influenza produced an unusually high proportion of cases that developed pneumonia, especially in young people. Normally, influenza causes only mild illness in young adults. The graph of death rate by age is shaped like a U, with high

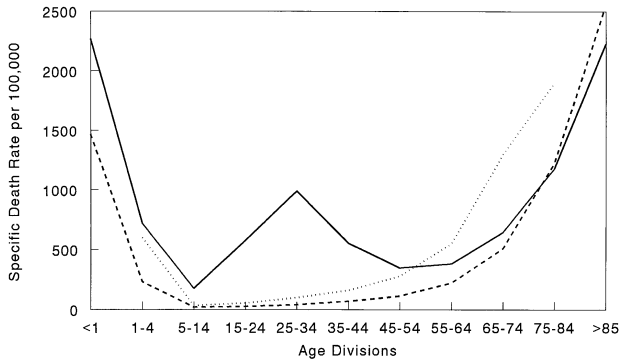

Figure 1. Influenza and pneumonia mortality by age, United States. Influenza and pneumonia specific mortality by age, including the pandemic years 1892 and 1918, and the average of the interpandemic years 1911–1915 is shown. Specific death rate is per 100 000 of the population for each age division. Key: 1892 pandemic (dotted line), 1918 pandemic (solid line), average of interpandemic years 1911–1915 (dashed line) [22–24].

rates for the very young and the very old [20]. The graph for the 1918 flu, in contrast, is shaped like a W, with a steep peak for 15–45-year olds [21–24] (*figure 1*). The death rate from pneumonia cases was somewhat higher than in other pandemics, suggesting that the pneumonic complications were more serious [21], but not dramatically so. These two facts (more pneumonia in general, and more pneumonia among young adults in particular) need to be examined separately.

The seriousness of an influenza infection is determined by how many (and which) cells the virus infects before being stopped by the body's immune system. A virus that can infect more cells, either because it replicates exceptionally well or because it infects cells not normally targeted by influenza will cause a more severe infection. However, specific antibodies can stop even an exceptionally virulent virus. The severity of a pandemic, then, will be determined by the inherent virulence of the virus and by the immune status of the population. For example, the severity of the 1968 pandemic was muted by widespread immunity to its neuraminidase protein, which it shared with its predecessor.

If we assume that the 1918 virus was more virulent than other pandemic viruses, we might expect that the normal U-shaped curve would be shifted upward; the death rate would be uniformly higher across all age groups. In fact, this is true only for parts of the curve; for those under 15 years of age and for those between 41 and 60. For 15–40-year olds, where we would expect the rate to be very low, the curve instead forms a distinct peak. In the elderly, the death rate falls below that experienced in the 1892 pandemic, even lower than their average influenza death rate in the non-pandemic years of 1911–1917 (*figure 1*). The unusual shape of the 1918 death rate curve suggests that while its agent may have been exceptionally virulent, that fact alone can not adequately explain its impact.

Our understanding of the 1918 flu would be incomplete if it did not also explain the unique age distribution of those deaths. Simonsen et al. noted that younger age groups account for a higher percentage of excess deaths in all pandemics, with older age groups accounting for increasingly more excess mortality in subsequent years [20]. Whereas all age groups are similarly vulnerable to a new virus, young people appear to acquire more complete immunity from their initial encounter with the pandemic virus. In subsequent years the young are thus better protected from the virus' descendants. In 1957 and 1968, 36 and 48%, respectively, of the excess deaths were in people under 65 years of age. Most of these excess deaths were in 45–64-year olds. In 1918, fully 99% of excess deaths were among people under age 65. The majority of these were between 15 and 35 years of age; the death rate of 45–64-year olds being only somewhat higher than in other pandemics. The pattern of greater pandemic vulnerability in the young was maintained, but the degree of the shift in vulnerable age groups was more marked. In the United States, if the 15–45-year-old age group had experienced its usual low death rate, the number of deaths would have been reduced to 285 000, about double the average number of deaths per year from influenza in those pre-antibiotic years.

What was it about the 15–35-year olds of 1918 that made them particularly susceptible to the pandemic virus? That many of them were soldiers living in miserable conditions is not sufficient explanation; the same death rates were seen in young people unaffected by the war. Did the robust immune systems of young adults overreact to the novel virus? One might expect such a phenomenon to contribute to higher death rates in this age range in all pandemics. The sharpness of the mortality peak in the 15–35-year range also argues against such an explanation, since the decline of the immune system is a gradual process. Furthermore, the pathological evidence does not point to a massive immune response; in many cases death came too quickly for a naïve immune system to have responded. However, differences in immune status could have been a factor in the odd death rate curve of 1918. If the virus replicated extremely efficiently, the difference between a mild case and full-blown pneumonia could be made by an only slightly slower immune response.

Hemagglutinin subtypes can recycle in the human population after enough time has passed that the majority of the population has no immunity [25]. The lower death rates among the elderly in 1918 might indicate that an H1 subtype virus was circulating before 1850. Since the elderly experienced a lower than expected death rate (unlike 1957 and 1968) perhaps the 1918 virus was more similar to the previously circulating strain than was the case in the later pandemics. Those people between 15 and 45 years of age in 1918 would have been exposed to a different set of influenza viruses than people of other ages, perhaps resulting in an anti-influenza immune status particularly ill-suited to the virus of 1918.

3. Molecular analyses of the 1918 virus

Even though contemporary observers were unable to isolate the causative agent of the pandemic, many detailed

studies of the epidemiology and pathology of the disease were carried out. Subsequently, as more was learned about influenza viruses and the origins of pandemics, some characteristics of the 1918 virus could be deduced. As the sequence of the virus is determined, it does not emerge into a vacuum. There is a body of historical and biological knowledge with which analysis of the sequence of the 1918 virus must be consistent. Answering questions about the origin and virulence of the 1918 virus requires integration of what is known about the history of the pandemic, influenza virus biology and the actual characteristics of the virus as they are revealed through sequencing.

Advances in the techniques of molecular biology have allowed us finally to get a closer look at the virus that caused the 1918 pandemic [11–13]. From preserved autopsy samples of two US soldiers and from the frozen lungs of an Inuit woman, fragments of the deadly virus have been isolated, copied and analyzed. The strains from these three cases have been named A/South Carolina/1/18, A/New York/1/18 and A/Brevig Mission/1/18. For the first time, it has become possible to test hypotheses about where the 1918 influenza virus came from, and what made it so deadly.

4. Hemagglutinin gene

In 1918, while the clinical similarity between annual, localized outbreaks of influenza and periodic global pandemics was noted, there was uncertainty as to whether such different phenomena could be caused by the same agent. The conditions for the emergence of a pandemic influenza strain had been identified: the virus must have a hemagglutinin (HA) protein distinct from the one currently prevailing, this HA subtype cannot have circulated in humans for 60–70 years, and the virus must be transmissible from person to person [26].

The HA protein is expressed on the surface of the virus. It must be cleaved by proteases to become active, whereupon it binds to receptors on host cells and initiates infection. Antibodies against the HA protein prevent receptor binding and are very effective at preventing re-infection with the same strain. The explosive, worldwide spread of influenza that characterized the 1918 pandemic suggests that at least the HA of that virus was novel to humans. Both the 1957 and the 1968 pandemic viruses had HA proteins closely related to those found in avian influenza viruses. Serological studies carried out in the 1930s showed that people born before 1918 had antibodies that neutralized swine H1N1-subtype influenza, while people born after that date did not.

Influenza virus infection requires binding of the HA protein to sialic acid receptors on the host cell surface. The HA receptor-binding site consists of a subset of amino acids that are invariant in all avian HAs but vary in mammalian-adapted HAs. The 1918 pandemic virus was an H1 subtype influenza. To shift from the avian receptor-binding pattern to that of swine H1s requires only one amino acid change, E190D [12]. All three 1918 cases have the E190D change. In fact, the receptor-binding site of one of the 1918 cases (A/New York/1/18) is identical to that of A/Sw/Iowa/30. The other two 1918 cases have an additional change from the avian consensus, G225D. Since swine viruses with the same receptor site as Sw/Iowa/30 bind both avian- and mammalian-type receptors, A/New York/1/18 probably also had the capacity to bind both. The change at residue 190 may represent the minimal change necessary to allow an avian H1-subtype HA to bind mammalian-type receptors, a critical step in host adaptation.

Certain influenza subtypes (H5 and H7) have insertional mutations of extra basic amino acids at the H1-H2 cleavage site which make them extremely virulent by expanding their tissue tropism [27]. The 1918 HA gene did not have this mutation [11].

The 1918 HA is more closely related to avian strains than any subsequent mammalian HA [12]. Of the 41 amino acids that have been shown to be targets of the immune system and subject to antigenic drift pressure in humans, 37 match the avian sequence consensus, suggesting that there was little immunologic pressure on the HA protein before the fall of 1918. Another mechanism by which influenza viruses evade the human immune system is the acquisition of glycosylation sites to mask antigenic epitopes. Modern human H1N1s have up to five glycosylation sites in addition to the four found in all avian strains. The 1918 virus has only the four conserved avian sites.

5. Neuraminidase gene

The complete sequence of the 1918 neuraminidase (NA) gene has also been determined [13]. In many ways analyses of the 1918 NA sequences give results similar to that of the 1918 HA. As with HA, the functional and antigenic sites of A/Brevig Mission/1/18 (Brevig/18) NA closely resemble avian isolates. The 1918 virus had an N1 subtype NA. The 15 conserved amino acids making up the active site of the molecule are retained, as are the seven glycosylation sites found in all avian strains. Twenty-two amino acids have been identified as antigenic in the N2 subtype [28]. Of the homologous amino acids in N1, 15 have shown variation in human strains. Brevig/18 matches the avian consensus at 14 of the 15 residues, suggesting little or no antigenic pressure on the protein before 1918. Human strains from the 1930s show extensive drift at these sites.

Several early human strains have deletions of 11–16 amino acids in the stalk region of NA that may affect the activity of the protein [29]. The 1918 strain does not have a stalk deletion, suggesting that the various deletions found in early human strains are likely to be artifacts of their extensive culture in various hosts.

Certain mouse-adapted H1N1 influenza viruses have a mutation leading to the loss of a glycosylation site at residue 146, the absence of which contributes to the extended tissue tropism and neurotropism [30–33]. The 1918 viral NA gene does not have this mutation.

Neither of the two mutations previously characterized in the HA and NA proteins of virulent avian or mouse-adapted influenza strains were present in the 1918 virus

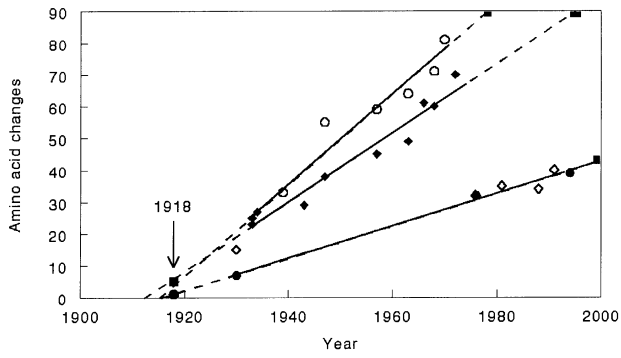

Figure 2. Change in HA and NA proteins over time. The number of amino acid changes from a hypothetical ancestor was plotted versus the date of viral isolation for viruses isolated from 1930–1993. Open circles, human HA; closed diamonds, human NA; closed circles, swine HA; open diamonds, swine NA. Regression lines were drawn, extrapolated to the x-intercept and then the 1918 data points, closed square, 1918 HA, closed circle, 1918 NA were added to the graph (arrow).

[11–13]. It is thus unlikely that the 1918 virus was either neurotropic or pantropic. These results corroborate the extensive pathologic examinations performed during the 1918 pandemic which demonstrated that significant pathology was limited to the lungs [15, 34].

6. Phylogenetic analyses

Phylogenetic analyses of the 1918 HA and NA genes allow us to place them within the context of a wide range of H1 and N1 subtype genes. Consistently, the 1918 HA is found within and near the root of the mammalian clade [12]. Its placement is compatible with its being the ancestor of all subsequent human and swine H1 subtype strains. Some analyses place the 1918 HA in the human clade and some in the swine clade, suggesting that it shares characteristics of both. The HA proteins of human influenza viruses are subject to substantial immune pressure; HAs that have acquired mutations changing or masking antigenic sites have a selective advantage for spread in humans. Therefore, by the time influenza strains were isolated from humans in the 1930s, many antigenic sites had drifted from the 1918 sequence. In swine the substitution rate is lower. As a result, the earliest swine influenza strain, isolated in 1930, in many respects resembles the 1918 strain more closely than the 1930s human strains.

If the substitution rates of human and swine influenza are projected back in time, the 1918 HA falls near the intersection of the human and swine lines (*figure 2*). This, in turn, is consistent with the historical record wherein concurrent outbreaks of influenza in swine and humans were reported in the US, Europe and Asia. In the US, the disease became established in swine and has recurred yearly since 1918.

The placement of the 1918 HA at the root of the mammalian clade is compatible with the historical record and with what is known about influenza evolution. However, it does not pinpoint where the HA gene of the 1918 virus came from or when it began circulating in humans. Both the 1957 and 1968 pandemic strains had HA proteins that were very similar to those found in wild birds. Since these proteins had never circulated in humans, they were antigenically novel and able to spread quickly through the human population. The rapid spread of the 1918 flu suggests that it also had acquired a novel HA.

In spite of its many avian characteristics, the 1918 HA is nevertheless phylogenetically distinct from current avian H1s. It is possible that the HA involved in the pandemic did not pass directly from an avian source to its pandemic form but rather spent some unknown amount of time adapting in a mammalian host. Alternatively, current avian strains may have drifted substantially from their 1918 form and no longer closely resemble the HA that made the jump to a human virus. Without samples of avian viruses from 1918, it is difficult to choose between these possibilities.

Phylogenetic analyses of the NA gene show that of all mammalian isolates, the 1918 sequence is the most closely related to avian isolates, but also suggest that the 1918 sequences share enough characteristics with mammalian isolates to distinguish them from the avian clade [13]. The placement of the Brevig/18 NA nucleotide sequence in the phylogenetic trees is usually within and near the root of the mammalian clade, suggesting that the 1918 NA is very similar to the ancestor of all subsequent swine and human isolates. At the same time, and in contrast to the results with HA, phylogenetic analyses of the NA protein sequence place Brevig/18 within the avian clade. In these cases branch lengths are very short and bootstrap values are low, suggesting that there are not enough differences among the sequences to place them unambiguously.

Phylogenetic results and structural characteristics suggest that the Brevig/18 NA sequence is intermediate between avian and mammalian sequences, and are consistent with the idea that the 1918 pandemic virus acquired its NA gene directly (with little modification) from avian viruses. Nevertheless, Brevig/18 NA differs at 26 amino acids from its nearest known avian relative (A/Dk/Alberta/76). By contrast the 1957 pandemic N2 and the N1 from the 1997 Hong Kong H5N1 outbreak differ by only 18 and 2, respectively, from their nearest avian relatives. Again, either avian sequences have drifted away from their ancestral sequences over the past 80 years, or the 1918 genes acquired mammalian-specific changes in a mammalian host in the years preceding the 1918 pandemic. That the ultimate source of the 1918 NA was avian is supported by the phylogenetic analyses, but the precise path of the gene from its avian source to its pandemic form cannot be determined by sequence alone. As with HA, phylogenetic analyses indicate that the 1918 NA is the likely common ancestor to subsequent human and swine H1N1 lineages (*figure 2*).

7. Origin of the pandemic virus

If one of the requirements for a pandemic influenza virus is that it have, at least, a novel HA protein, another is

that is must be readily transmissible from person to person. A pandemic virus faces the twin challenges of being 'new' to its host, while being supremely well adapted to it. This condition has been fulfilled in recent pandemics by reassortment: combining surface proteins novel to humans with human-adapted internal proteins. A trade-off is implied, the more avian genes, the less recognition by the human immune system, but probably the less well adapted to growth in human cells. The 1997 outbreak of H5 influenza in Hong Kong may be a case in point. It was an entirely avian virus and, while it caused severe illness in several people, it apparently spread extremely poorly, if at all, from human to human [35–37]. We have obtained sequences from three of the six RNA segments of the 1918 virus that code for the internal proteins of the virus: NS, NP and MA ([11] and unpublished data). The sequences of the all the genes examined appear to be more closely related to old human and swine strains than to avian strains.

The difficulty in determining whether any or all of these genes shifted in 1918 is that the virus it replaced is not available. Our data confirm an H1N1 shift around 1918, but cannot determine with the precision the date when these genes entered the human population. If all human influenza viruses ultimately derive from avian sources, the oldest human strain will necessarily be more closely related to avian strains than all subsequent strains. In the case of HA and NA, the 1918 genes do not seem to be as closely related to avian strains as were the surface proteins of the 1957 and 1968 pandemics. For the other genes, even with sequence in hand it will be difficult to determine whether a gene was new to humans in 1918 or had entered the human population earlier, although phylogenetic analyses may provide clues.

8. Conclusion

In many respects, the 1918 influenza pandemic was similar to other influenza pandemics. In its epidemiology, disease course and pathology, the pandemic generally was different in degree but not in kind from previous and subsequent pandemics. However, there are some characteristics of the pandemic that appear to be unique. Mortality was exceptionally high, ranging from five to twenty times higher than normal. Clinically and pathologically, the high mortality appears to be the result of a higher proportion of severe and complicated infections of the respiratory tract, not with systemic infection or involvement of organ systems outside the influenza virus's normal targets. The mortality was concentrated in an unusually young age group. Finally, the waves of influenza activity followed on each other unusually rapidly, resulting in three major outbreaks within a year. Each of these unique characteristics may find their explanation in genetic features of the 1918 virus. The challenge will be in determining the links between the biological capabilities of the virus and the known history of the pandemic.

Acknowledgments

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