Chapter 3
Swine and Avian Influenza Outbreaks in Recent Times

3.1 Introduction

Zoonotic influenza continues to pose serious threat to the welfare of the global population, and it is predicted by some experts that the next major influenza pandemic will be of avian origin from Asia. To prepare for the future, we have to learn from the past events, and this involves analysis of recent occurrences in influenza activity, including epizootic outbreaks. Rapid transportation by airplanes and a much larger global population than existed just over a century ago, during the 1918 Spanish influenza pandemic, may result in greater human suffering. Modernization of the global healthcare facilities and effective medicines and vaccines will likely prevent a repeat of the 1918 explosive mortality rate, and the brunt of a virulent influenza pandemic will be borne by the poorer countries, the debilitated elderly, pregnant women, and the growing number of people with immunosuppression.

3.2 Virology

The influenza viruses are enveloped viruses of *the Orthomyxoviridae* family, with influenza A and B causing annual seasonal excess morbidity and mortality and influenza C a milder respiratory illness [1]. Only influenza A viruses are true zoonotic agents responsible for influenza pandemics, and influenza B and C are primarily human pathogens, but influenza C occasionally infects pigs and dogs [2]. The morphology of the influenza viruses includes segmented, single-stranded, negative-sense RNA genomes, surrounded by nucleoprotein envelope and covered with surface projections or spikes [1]. These surface spikes are glycoproteins with hemagglutinin [HA] or neuraminidase [NA] activity that are the main targets of the host humoral immune response. The influenza A viruses can be subtyped according to the antigenic nature of their surface glycoproteins, with 16–17 HA and 9–10 NA identified to date.

The HA serves as the receptor-binding protein and facilitates fusion of the viral envelope with the host cell membrane [3]. NA is responsible for assisting virus entry into cell by mucus degradation [4] and release and spread of the progeny virions [4]. There are eight RNA segments within the viral genome, encoding 10–11 proteins.

Influenza A and B viruses frequently undergo antigenic variation of the surface glycoproteins [HA and NA] that allow the viruses to evade human neutralization from previous exposures and vaccinations. Variation in the viruses is caused by accumulation of point mutations in the HA and NA genes, antigenic drift [5, 6]. A variety of mutations including substitutions and deletions result in genetic variation during antigenic drift. The gradual accumulation of amino acid changes that occur with antigenic drift on the HA and NA sites allows the virus to survive, due to ineffective neutralization, and replaces existing strains as the predominant circulating virus in the population, to cause yearly outbreaks [1]. The propensity of influenza viruses for multiple mutations and rapid antigenic variation may be due to evolutionary effect of selective pressure for self-preservation. High error rate or mutations during genomic replication are typical of RNA viruses, as the RNA polymerase lacks proofreading activity, and the segmental influenza virus genome facilitates reassortment between different viral strains that infect the same cell [6–8].

A major antigenic variation, called antigenic shift, that usually precedes a pandemic occurs only in influenza A viruses and by a different mechanism. Antigenic shift results in the introduction of a new HA, with or without a new NA, to introduce a new virus to which the population is naïve, lacking any or even partial immunity [1, 6]. This pattern of replacement of previous HA and NA in new subtypes of influenza A viruses is associated with emergence of pandemic influenza outbreaks over the last century. Antigenic shift may occur by at least two different mechanisms. A zoonotic influenza virus can be transmitted from an animal reservoir without reassortment, usually by adaptation to a mammalian or human receptor site by mutation [1, 6]. The other mechanism is by reassortment, when two or more viruses coinfect the same cell and exchange one or more RNA segments to produce new progeny virus with new antigenic HA [with or without new NA] and new biological properties [7]. Swine is commonly believed to be the "mixing vessel" for genetic reassortment, as they are susceptible to infection with both avian and human strains and swine strains of influenza viruses [9]. The Asian influenza pandemic of 1957 and the Hong Kong influenza pandemic of 1968 were the result of antigenic shift with reassortment of genetic material during dual infection with circulating human influenza strains, H1N1 in 1957 and H2N2 in 1968, and avian influenza strains, probably with pigs as the mixing vessel [10, 11].

3.3   Ecology and Host Tropism

Influenza A viruses can infect several animals including humans, birds, pigs, horses, cats, and marine mammals such as seals and whales [1, 6]. Most influenza viruses are restricted to specific hosts, but some strains can circulate among several animal

species, i.e., H1N1 and H3N2 viruses are endemic in humans, birds, and pigs [12]. The primary determinant of host tropism and transmission is directly related to the specificity and affinity of the viral HA for the host receptor. Sialic acid is the receptor for influenza viruses that binds to the viral HA. Avian influenza viruses preferentially bind to sialic acid molecules with specific side chains with α-2,3-linkages and mammalian viruses to α-2,6-linkages [6]. Human-adapted seasonal viruses such as H1N1 and H3N2 have high affinity for α-2,6-linked sialic acid [SA], which are expressed on the epithelium of the upper respiratory tract [URT] of humans [12]. Avian influenza viruses bind preferentially to galactose-linked α-2,3-SA which is found abundantly in the URT and gut of birds, but is also found in the lower respiratory tract epithelium of humans [12]. Hence, highly pathogenic avian influenza viruses [HPAIVs] not well adapted to human receptors can cause limited avian to human transmission with viral pneumonia by this mechanism. The tracheal epithelium of pigs contain receptors with both α-2,3-SA and α-2,6-SA linkages and be infected simultaneously with avian and human [mammalian] influenza viruses that predispose to reassortment of zoonotic and human strains [12]. See Fig. 3.1 for ecology, cycle of avian, and swine influenza A.
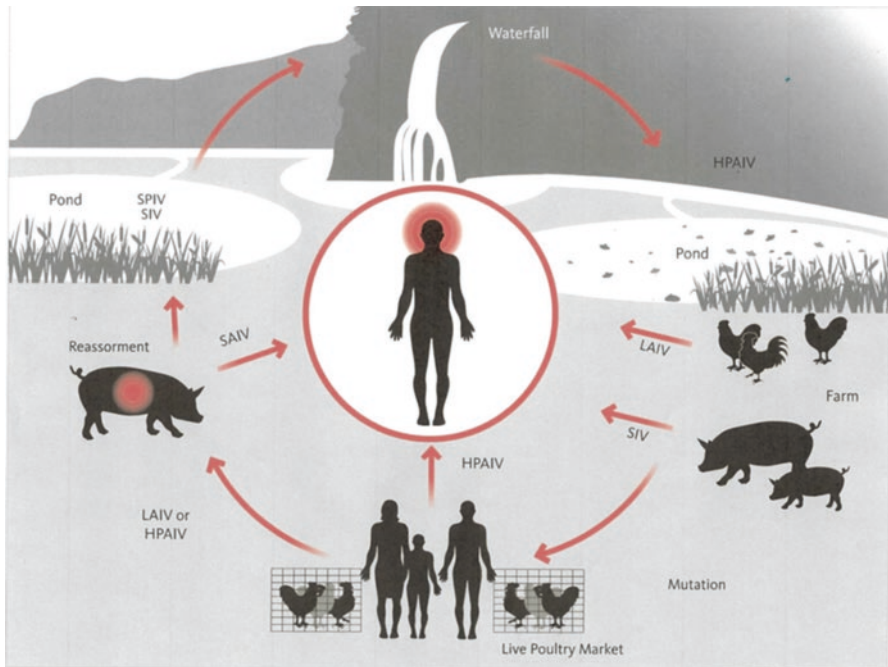

Fig. 3.1 Biological cycle of avian and swine influenza A. *LPAIV* low pathogenic avian influenza virus, *HPAIV* highly pathogenic avian influenza virus, *SIV* swine influenza virus, *SAIV* influenza virus with genetic elements from avian and swine influenza

3.4   Swine Influenza Viruses of the Twenty-First Century

The spread of influenza zoonotic viruses in animals and humans is primarily related to the interface and transmission between birds, pigs, and humans. Swine influenza viruses [SIVs] was first isolated from pigs in 1930 [13], although clinical disease resembling influenza was noted in pigs in the midwestern United States [US] in 1918, coinciding with the Spanish influenza outbreak [14]. Since then swine influenza [SI] has been recognized worldwide in the pig industry as a significant problem. The clinical signs of influenza in pigs are similar to those in humans with high herd morbidity [nearly 100%] and low mortality [<1%], but pneumonia can occur and recovery usually after a week [15]. The epizootic pattern of SI is similar to that in humans with outbreaks in late fall and early winter. The predominant SIV circulating in North America from the first isolate in 1930 to 1998 was caused by the swine H1N1 lineage [SH1N1], but there was low level of human subtype H3 virus also circulating in pigs [16]. In 1998 a severe SI outbreak occurred in pig farms in several states of the US, this was subsequently attributed to a new SIV subtype H3N2 [15]. Within a year there was widespread circulation of the SH3N2 virus in pig farms across the US, containing gene segments similar to those of human influenza subtype and the classic SI subtype [double reassortment], and another strain with the gene segments from human, swine, and avian lineages, triple reassortment [17]. The triple reassortment SIV circulated more efficiently than the double reassortment virus in the pig population. Over the years in less than a decade, multiple reassortment SIVs have been identified in North America, including H3N2 genotypes, H1N2, reassortment [r] H1N1, and H3N1 [15]. In 2006 humanlike H1 viruses genetically and antigenically distinct from the classic swine H1 lineage were identified in pigs in Canada and subsequently spread across the US swine farms as H1N1 and H1N2 viruses [15, 18].

3.4.1   Cross-Species Transmission and Mixing Vessel Concept

Zoonotic influenza A viruses are predominantly species specific, and although cross-species transmission of influenza viruses occurs fairly frequently, they are usually self-limited and rarely maintained in the new host. There are numerous examples of cross-species transmission of influenza virus from avian to mammalian species and intra-mammalian cross-species infection [15]. Specific subtypes of influenza viruses differ in their ability to cross the species barrier, and viral and host factors are important in the transmission. The segmental nature of the influenza genome is considered important in the viral evolution and cross-species transmission. Host range restriction of species transmission is governed by several viral proteins, but HA is critical to bind to host receptor to allow invasion and replication. Avian influenza viruses preferentially bind to α-2,3-SA receptors in intestinal epithelial cells, whereas human influenza viruses HA bind more favorably to respiratory epithelial receptors with α-2,3-SA [19]. Thus, avian influenza viruses usually cannot replicate effectively in humans, and birds are less susceptible to human

viruses. NA also contributes to the virus-species host specificity as efficient growth of influenza A virus depends on the balance between HA receptor-binding affinity and the NA receptor-cleaving activity [9]. The viral polymerase basic protein-2 is important in virus replication and is a host range determinant [20].

The concept that swine could be a "mixing vessel" for reassortment of influenza viruses was proposed by Scholtissek et al. [21] in 1985, as pigs could be dually infected with human and avian influenza viruses. This is related to the presence of both receptor types found in the respiratory tract of pigs [22]. Documentation of primary avian influenza viruses in swine has been reported over the years in different regions of the world: in European swine in 1979, pigs from China and Asia multiple times, and in Canadian swine in 2000–2004 [9]. Wholly human influenza viruses in pigs had also been well documented a few times in Taiwan and China [23], and pig-pig transmission of human H1N1 viruses has been reproduced experimentally [24]. Prior to 2005 sustained circulation of human influenza viruses was uncommon in swine herds, but since then swine viruses containing human-origin H1 and H1N2 gene segments have become established in the US [9].

3.4.2 Reassortment of Influenza Viruses in Pigs

It has been proposed that the influenza A viruses responsible for the 1957 and 1968 human pandemics were the result of avian, human, and swine viruses reassortment, but direct evidence was lacking. The PB1 gene of influenza A virus, involved with initiation of transcription and chain elongation with other viral polymerase gene products [25], was introduced from avian species into the human pandemic strains [1957 H2N2 and 1968 H3N2], and this avian PB1was also found in pig viruses [26]. Genetic reassortment between avian and human H3N2 viruses has occurred in European pigs [27], and novel reassortment viruses were transmitted to children in the Netherlands [28]. A similar reassortment H3N2 virus was isolated from a child in Hong Kong [29].

Since 1998 double [human/swine] and triple [avian/human/swine] reassortment viruses, H3N2, H1N2, rH1N1, and H3N1, have emerged in US pigs [9]. The predominant viruses circulating in US swine are these triple reassortment H3N2, H1N2, and H1N1 viruses. With the advent of the twenty-first century, avian H9N2 and existent human H3N2 influenza viruses were co-circulating in pigs of southeastern China [30]. Subsequently, double reassortment H3N2 viruses containing human viral genes [HA and NA] and avian genes [polymerase, matrix, and nonstructural proteins] and triple reassortment H3N2 viruses carrying human, avian, and swine viral genes have emerged in pigs in China [31].

In central US a unique H2N3 influenza virus was recovered from pig farms in 2007 [32], with HA and NA sequences similar genes to avian influenza viruses [H2N3 and H4N3] and genes from US swine influenza viruses. This swine H2N3 virus with avian origin surface glycoprotein was already well adapted to the mammalian host. Of concern was that the H2 influenza viruses were absent from the

human circulation since 1968, and individuals born subsequently would have had little immunity to this subtype [19], thus posing a pandemic risk to a large nonimmune human population. The HA mutation was identical to the initial human influenza virus isolates found at the beginning of the 1957 H2N2 pandemic [9].

3.4.3   Transmission of Swine Reassortment Viruses to Humans

Although some experts believe that the Spanish influenza pandemic of 1918 was caused by a reassortment swine H1N1 virus, this is controversial with no direct supporting evidence and others contend that it is of avian origin. The first swine influenza virus isolated from human was in 1974 [33], and prior to 2009 there were only sporadic cases reported. Myers et al. [34] subsequently reviewed 50 cases of zoonotic swine influenza reported up till 2005. Cases were reported from the US, Europe, Russia, Canada, and Hong Kong. Most cases [37 subjects] were civilians but there was a localized outbreak of 13 cases in the military, Fort Dix, New Jersey. Swine exposure was reported in 61% of civilians, and the case fatality rate was 14%, 7 of 50 infected. The predominant influenza A viruses were H1N1 subtype with only 4 H3N2 subtype.

In 2009 a swine-derived H1N1 influenza A reassortment virus caused a moderately mild pandemic, the "Mexican" influenza pandemic. The first confirmed cases of the pandemic virus appeared in Mexico in February 2009, followed by cases detected in California in March–April that year [35]. The speed of the pandemic was rapid by June 2009; 73 countries had reported 26,000 confirmed cases. The initial outbreak in Mexico was the most worrisome, as 6.5% of hospitalized patients became critically ill and 41% of these patients died [36]. By August 2010, nearly all countries in the world reported confirmed cases of the pandemic H1N1 influenza, but the global outbreak started to wane. Although experts considered the 2009 pandemic a mild outbreak, there was marked variation in the severity of the disease in different regions of the world [37]. Unlike seasonal influenza outbreaks, and similar to the 1918 pandemic, older adults fared relatively well, and excess mortality and adverse outcome were greater in children, young adults, and pregnant women [35]. Estimates of the influenza-related deaths worldwide, 123,000–203,000, were similar to that of mild seasonal influenza [37]. Baseline pre-existing immunity at the start of the 2009 pandemic was nonexistent in children and very low in those born after 1980, but greater in older adults [38, 39].

The influenza A H1N1 2009 pandemic virus was first detected in Canadian pigs in May 2009 and subsequently in pigs from 14 countries in the Americas, Europe, and Asia [40, 41]. Since then new reassortment events with endemic swine influenza strains were reported in pigs in Hong Kong [42], Italy [43], Germany [44], and the US [45], derived from the 2009 swine H1N1 influenza virus. In the US nine reassortment viruses representing seven genotypes were found in commercial pig farms [45]. The pandemic strain of 2009, H1N1pdmo9, was antigenically related to the 1976–1977 swine influenza virus in the Fort Dix outbreak; and the

genetic makeup consisted of a number of reassortments between avian, human, and swine viruses [46].

Since the influenza pandemic of 2009, H1N1-pdm09 has continued to circulate in various regions of the world during seasonal influenza activity. Influenza A viruses recovered in the latter part of March–April, 2015, from 84 countries were reported by WHO to be H1N1-pdm09 in 48.5% of isolates [WHO, Influenza Update no. 235, April 21, 2015]. The largest outbreak of influenza A [H1N1-pdm09] in recent years has been reported in India. Since the 2009 pandemic, the H1N1-pdm09 has replaced the previous seasonal H1N1 and became established in the human population. In India H1N1-pdm09 has recently caused a localized outbreak in the northern region in 2014–2015, with at least 22,240 cases and 1194 influenza-related fatalities [47]. The influenza HA sequences from viruses isolated in India indicate that the virus has gradually evolved since 2009 and acquired mutations in the H1 antigen sites and linked to enhanced virulence and appears to be antigenically distinct from the current vaccine containing 2009 [Ca10109] H1N1 viral HA [48]. In the US 13 cases of infection with novel triple reassortment swine-origin, influenza A [H3N2], variant virus occurred between 2011 and 2012 and were mostly related to agriculture fairs [49].

3.5 Avian Influenza in the Modern Era

Although avian influenza A viruses may have caused human epidemics for centuries, this has not been well documented. In 1557 and 1580, influenza pandemics, called "chicken malady" in German because the human cough sounded like sick chickens, were not preceded or concurrent with poultry outbreaks to link the events with avian influenza [50]. However, it is possible these outbreaks could have been related to low pathogenic avian influenza viruses [LPAIVs] without symptomatic disease in poultry. The first epizootics of avian influenza in poultry were described in Northern Italy in 1789, and they were not associated with human outbreaks [51]. However, highly pathogenic avian influenza viruses [HPAIVs] became well known to veterinarians around the end of the nineteenth century, after description of the "fowl plague" by an Italian scientist [51]. Although the origin of the 1918 Spanish influenza pandemics is still not fully resolved, the virus has avian-like genome probably derived from HPAIV a decade before [52].

Avian influenza is now considered by experts to be the greatest threat to global public health to arise from animals. Before the end of the millennium, HPAIV was linked to poultry but occurred rarely with self-limiting course. Since then a marked increase in avian influenza outbreaks has occurred worldwide. It has been estimated that avian influenza outbreaks have increased 100-fold with 23 million birds affected between 1959 and 1998 and over 200 million from 1999 to 2004 [53]. Poultry outbreaks continued to emerge even in Europe and North America in the first decade of the twenty-first century, with substantial damage and cost to the poultry industry and with sporadic human infections [51].

The natural reservoir hosts of avian influenza A viruses are wild waterfowls, such as ducks, geese, swans, gulls, waders, and others, and typically are asymptomatic in the birds or cause mild disease [54]. Thus, wild waterfowls are natural hosts for LPAIV which are transmitted to domestic birds and mammals by fecal-oral route through contamination of water, soil, and the environment. Poultry also have subclinical or mild respiratory disease, but they represent the main transmitters to humans. In domestic fowls such as chickens and turkeys, LPAIV of H5 and H7 subtypes may evolve to become more virulent as HPAIVs with lethal effect and can be transmitted via fecal-oral route or through the respiratory secretions. LPAIVS and adapted variants [HPAIVs] can cause respiratory disease in mammals and humans of varying severity with respiratory transmission [51]. Cross-species transmission had resulted in human infections with LPAIV H9N2, H7N2, H7N3, and H7N7 [51]. HPAIVs have rarely been transmitted from poultry to other species, but in the past decade or more, they have caused increasing respiratory and systemic infections in humans and other animals. Cross-species transmission of HPAIVs to humans had occurred with H5N1, H7N3, and H7N7 [55–57]. In most cases transmission from poultry to humans occurred via the respiratory or ocular route, but some strains of the HPAIV H5N1 may have been transmitted by both respiratory and oral routes to mammals. To date avian influenza viruses have limited or no human-to-human transmission capability. HPAIV infections manifestations are more atypical than regular influenza and may include gastrointestinal and neurological symptoms/signs besides the usual respiratory disease with H5N1 and ocular disease [conjunctivitis] with H7 subtypes [51].

Domestic ducks are especially prone to a large diversity of LPAIV infections from consumption and contact with surface water shared with wild waterfowls [58]. Terrestrial birds [chickens] associated with dry environment may be infected via respiratory route from droplets or aerosols and fecal-oral route through contaminated fomites [59]. Humans and other mammals are infected by avian influenza viruses primarily through the respiratory tract with inhalation of droplets, fomites or aerosols from domestic fowl, or self-inoculation accidentally from the contaminated hands. Infection by digestion is unusual in mammals but has been demonstrated to occur with HPAIV H5N1 in ferrets, mice, hamsters, and cats [60, 61]. Inoculation of the conjunctiva appears to an important means of bird-to-human transmission with the H7 subtype, due to preferential tropism for ocular tissues in human [62]. Direct inoculation of the upper respiratory tract or conjunctiva while swimming in contaminated water is also possible, and this has been documented Southeast Asia infection with HPAIV H5N1 [62].

3.5.1 Tissue Tropism

LPAIV preferentially infects the epithelial cells of the distal small bowel and the cloaca of waterbirds [53]. Trypsin-like proteases in the small intestine can cleave the HA protein resulting in localized infection. In poultry LPAIV primarily infect

epithelial cells of the respiratory tract of the trachea, bronchi, and the alveolar sacs. Extracellular proteases for cleavage of the HA proteins are present in the respiratory epithelium of poultry [53]. Experimentally, however, intravenous inoculation of LPAIV can result in replication of the virus in the kidney and intestinal epithelial cells of chickens [63].

HPAIV, which evolve from LPAIV in poultry by mutations, have a broad tissue tropism in domestic fowls. The nasal cavity and the respiratory epithelium are initially infected with submucosa and capillary invasion, with widespread dissemination via the circulation to infect the epithelial cells of numerous organs throughout the bird's body. This leads to severe avian illness and high mortality, as the brain, pancreas, heart, kidney, and skeletal muscle can be affected in poultry in acute infection [53]. Wild waterfowls are rarely infected with HPAIV, but since 2002 HPAIV H5N1 has spread from poultry to a wide range of wild bird species. The HA protein of HPAIVs possess multibasic cleavage sites that can be cleaved by intracellular proteases present in a diverse number of cell types in avian and mammalian species [64].

Wild birds become infected with HPAIV H5N1 via the respiratory epithelium initially, with replication and viremia and dissemination to variable number of organs depending on the species. In contrast to LPAIV the intestinal tract is not usually infected by HPAIV in wild birds. In most infected wild birds, the parenchymal cells are the main sites for H5N1 replication, and although the virus can spread to many organs, the main tissue tropism is in the brain besides the respiratory tract.

In humans and other mammals, LPAIV and HPAIV preferentially cause infection of the respiratory epithelium and especially of the lower tract. Infections of humans with LPAIV typically cause mild respiratory disease, including conjunctivitis for H7 subtypes, with resolution in 1–2 weeks [54]. Tropism of the influenza viruses is in large part determined by the receptor-binding affinity of the HA protein. Avian influenza viruses bind preferentially to receptor with α-2,3-SA to galactose, which are abundant in the intestinal and respiratory epithelium of domestic and wild birds but are also present in other tissues [heart, kidney, and brain] and endothelium in ducks and chickens [64]. Binding of avian influenza in humans occurs mainly in the lower respiratory tract where α-2,3-SA linkages are present focally in bronchiolar epithelial cells, type 11 pneumocytes, alveolar macrophages, acinar cells of the submucosal glands of the trachea and bronchi, and epithelial cells of the eye [65–67].

3.5.2   Highly Pathogenic Avian Influenza H5N1

The HPAIV subtype H5N1 was first described in Southeast Asia in 1996, and since then it has spread to at least 63 countries in Asia, Europe, Africa, and the Middle East [68]. Although the virus likely originated from poultry as a result of mutations of a LPAIV, it appears to have infected wild birds, which subsequently caused spillback infection to poultry in distant regions. Migrating aquatic wild birds were considered responsible for long-distance dispersal of the HPAIV H5N1 from Qinghai Lake [China] to Europe, Russia, and Africa [69]. The ancestor virus was initially

isolated from domestic geese in China, but the prime long-distance vector appeared to be the wild mallard ducks, as they showed abundant viruses excretion without clinical or debilitating disease [70]. Over 100 million birds have died from the infection either naturally or from culling to limit the spread of the disease. In Thailand which had seven waves of HPAIV H5N1 outbreaks, >62 million poultry have died and outbreaks in poultry were associated with increased infection in wild birds in the preceding months [71].

Genomic analysis of H5N1 isolates form birds and humans in 2005 showed two distinct clades from separate noncontiguous regions [72]. All the genes were of avian origin with no evidence of reassortment with human influenza virus. The human isolates were resistant to amantadine but were susceptible to the neuraminidase inhibitors [72]. Isolation of the first human HPAIV H5N1 occurred in a child in Hong Kong in 1997, and this raised fears of an impending H5N1 pandemic [73]. Subsequently only 608 human cases had occurred up to August 2012 from 15 countries [74]. Most cases were related to contact with poultry or poultry products, and occasionally from contaminated water and human transmission appeared to be rare [74]. No human cases were reported from Western Europe or the Americas. The high case fatality rate reported to the WHO of about 59% was most likely an over estimate from unrecognized mild infections or subclinical cases. A recent prospective serological epidemiology study from Egypt, where most cases of H5N1 were reported since 2009 from backyard poultry producers, found that most seroconverters were asymptomatic or had mild disease [56]. Thus the true case fatality rate is likely very low. Although HPAIV H5N1 continue to circulate in poultry, spill over infection in humans and other mammals have remained rare. Hence so far, the HPAIV H5N1 has not mutated to allow facile transmission from poultry to humans or human to human.

3.5.3   Emergence of Avian Influenza A H7N9

Over the past several years, other avian influenza A viruses of subtypes H6, H7, H9, and H10 have crossed the species barrier and caused mainly sporadic, nonfatal cases of human infections [55, 75–79]. Some of these cases were secondary to low pathogenic strains that caused disease in persons with immunosuppression. However, a H7N7 strain caused human conjunctivitis and a case of fatal respiratory distress syndrome in the Netherlands [79]. Of major global public health concern is the emergence of a novel avian influenza A H7N9 virus in China in March 2013. Within two months of its appearance in the human population, the cumulative number of human cases in China was almost three times as high as the number caused by the H5N1 outbreak during a similar period of time [80]. By June 2013, there were 132 symptomatic cases and one asymptomatic case with 40 attributable deaths [81]. Infection with the H7N9 virus has been associated with a high incidence of severe disease, with rapidly progressive pneumonia and multiorgan failure associated with cytokine "storm" or severe dysfunction [81, 82]. In a review of 139 confirmed cases in China that occurred in the first 9 months of the outbreak, 99% were hospitalized,

90% had severe pneumonia or respiratory failure, and 63% required intensive care and 34% of the total cases died [83]. The first cases of H7N9 influenza infection appeared in eastern China around the Yangtze River delta and subsequently spread to 12 regions in China along an avian migratory pathway [81, 84]. Most cases were associated with exposure to poultry [82%], but in four family clusters, non-sustained human-to-human transmission could not be excluded [83]. At the onset of the outbreak in eastern China, there was no apparent outbreak in poultry or in wild birds. Analysis of the virus showed all gene segments were of avian origin, and the H7 isolated virus was closest to that of H7N3 virus from domestic ducks in Zhejiang, but the N9 was closest to that of the wild bird H7N9 virus in South Korea [85]. The H7N9 virus has been isolated from live poultry and the environment of poultry markets; and case-control studies confirmed the association of human infection with visits to these markets [83]. Moreover, closure of live poultry markets have resulted in the reduction of confirmed H7N9 influenza cases. The H7N9 avian influenza virus potentially poses a high risk to human populations, which are naïve to the virus, as the virus has biological properties conducive to aggressive disease in mammals. Studies have confirmed that the H7N9 virus can bind to both avian and human receptor and it can replicate efficiently in several mammalian cell lines, including human lower respiratory tract epithelial cells and type 11 pneumocytes of alveoli [86]. H7N9 avian virus replicated to higher titer in human respiratory epithelial cells and respiratory tract of ferrets compared to seasonal influenza H3N2 virus and produced greater infectivity and lethality in mice compared to other genetically related virus [87].

3.5.4   Current Status of the Emerging Pathogenic Avian Influenza Viruses

Epizootic HPAIV H5N1 continues to circulate in poultry in several countries of the world, and the virus has become endemic in Indonesia and Egypt since 2006–2008 [88]. The largest number of poultry afflicted from 2003 to May 2015 were in Vietnam, Thailand, and Egypt [88]. Fifteen countries continued to report H5N1 infection in poultry for the first 5 months of 2015.From February 2003 to March 2015, there have been 826 symptomatic [severe] cases of human influenza with HPAIV H5N1 recognized from 16 countries with 440 fatal, resulting in a case fatality rate of 53% in these clinically recognized cases. However, these diagnosed cases likely represent only a fraction of the total infected human subjects. In the first 4 months of 2015, only five confirmed cases of H5N1 influenza infection have been reported from the Western Pacific Region [89].

A unique feature of avian influenza A H7N9 human outbreak has been the absence of preceding bird epidemics with die off of poultry or wild birds. Thus, H7N9 appears to be a LPAIV with asymptomatic or mild infection in domestic and wild birds. This is attributable to the absence of a multibasic cleavage site of the HA, which is a virulence marker in birds but not in humans [85]. Also previous infection

in poultry with a closely related LPAIV [H7N3] may have elicited cross-protection in birds [81]. Hence, the distribution of H7N9 in poultry and wild birds is poorly characterized and is only retrospectively recognized after human outbreaks. Thus the H7N9 avian virus potentially is a greater risk than the H5N1 to produce widespread epidemics in human populations because of its stealth. So far human infection occurrence has been limited to mainland China, including 19 cases diagnosed in Taipei [Taiwan], Hong Kong, and Kuala Lumpur in Malaysia [90]. As of February 2015, a total 571 confirmed human cases of H7N9 infection has been reported to WHO with 212 [37.1%] deaths [91]. However, the true case fatality rate appears to be much lower as only the severe cases are recognized clinically. A recent seroprevalence study from southern China in poultry workers found that 7.2% in spring and 14.9% in winter have been infected with the H7N9 influenza virus with subclinical or mild infection [92].

Overt clinical influenza infection with the avian H7N9 virus appeared in three waves starting in the winter to the spring and with the first wave in February–May 2013 [see Fig. 3.2]. Since February 2015, 20 additional confirmed human cases have been reported from China and with four deaths [http://www.who.int/csr/don/14-April-2015-avian-influenza-china/en]. The majority of reported cases have had exposure to poultry and overall the public health risk from the avian H7N9 virus has not changed. Thus, the virus has not mutated to cause efficient human-to-human transmission. Although the extent of transmission in poultry is unclear, the H7N9 virus has persisted in domestic fowls with a seasonal pattern similar to that of other avian influenza viruses, circulating at higher levels in cold weather compared to warm seasons.
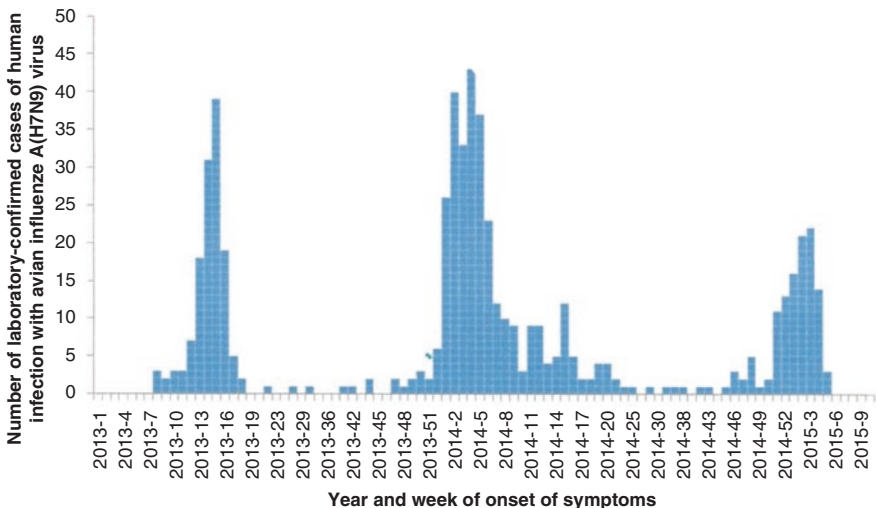

Fig. 3.2 Laboratory confirmed cases of human infection with influenza A [H7N9] virus by week of onset. World Health Organization, 23 February 2015

3.6   Strategies to Contain Zoonotic Influenza A

Measures to contain zoonotic influenza are already in place in many developed countries but are lacking or incomplete in many developing and middle-income countries. The exact sequence of events and break in control measures that led to the recent swine H1N1 pandemic is still unclear. The outbreak appeared to have originated in Mexico with the virus jumping the species barrier in local pig farms. Most developed countries have highly regulated, hygienic swine and poultry farms and backyard animal farms are not allowed. Culling of sick animals is regularly used to control pathogenic zoonotic infections. LPAIV would, however, avoid detection but hygienic infection control measures theoretically should limit cross-species transmission.

Live poultry or animal markets, which are common in Asia and other developing regions of the world, have been shown to be a primary source of avian influenza outbreaks in humans, and this cultural practice represents a major obstacle to the prevention of avian influenza and other zoonoses cross-species transmission. Disbanding these markets would be the only permanent solution, but so far countries such as China and others have not implemented any such measure. However, closure of these animal markets would not eliminate the risk for zoonotic influenza outbreaks in communities. These zoonotic viruses likely arise in farms, and the entire housing and transportation chain would be contaminated [90]. Moreover, LPAIVs would avoid detection in farms to alert farm workers and transportation staff of the risk of cross-species transmission. An example is the H7N9 outbreak, where the first positive farm detected with the virus was in Guangdong Province reported in March 2014, a year after recognition of human cases [90].

Local measures have been implemented in Hong Kong that reduced the epizootic spread of avian influenza and human outbreaks. In 1997 the H5N1 human outbreak was halted in Hong Kong after culling all poultry and restriction of importing chickens from mainland China only to farms with stringent biosecurity measures [93]. Where HPAIV outbreaks had occurred, all live poultry and poultry products from the affected province would be suspended for 21 days; and unaffected farms within 3 km of index farms would also have suspension for live poultry/products for 90 days. In live poultry markets, several control measures were instituted: segregation of poultry species to reduce the risk of genetic reassortment, regular cleaning of transport cages to limit trafficking of viruses from farms to markets and interrupt amplification of the viruses, and banning of overnight poultry storage in markets [93, 94]. Public education to avoid contact with poultry, proper hand hygiene after contact with poultry products, encouragement to purchase frozen chicken instead of live ones, and banning the possession of live poultry in the household were also implemented. These measures resulted in dramatic reduction in the isolation rate of HPAIV H9N2, and no local cases of avian H5N1virus infection has been identified in Hong Kong since 2007 [81].

3.6.1   Vaccines for Zoonotic Influenza A

It is generally considered that influenza vaccines are the most effective means of preventing or limiting the spread of influenza outbreak. However, there are several limitations to this approach, including our inability to predict the next pandemic strain of virus in order to produce sufficient vaccines in time to supply the global needs. This operational shortcoming was evident in the 2009 H1N1 influenza pandemic, as by the time 77 countries received adequate supply of vaccines [78 million doses] the outbreak was already waning [35].

Vaccines have also been used in animals since the 1990s to control highly pathogenic influenza epizootics, along with other methods. HPAIVs were first recognized as a cause of fowl plague in 1955, and since then 30 epizootics have occurred globally [95]. It is estimated that 58 billion poultry are raised each year in the world, and fowl plague may have affected only a fraction of about 250 million birds per year. Most HPAIV epizootics involved single countries, and only two have embroiled multiple countries, H5N1 since 1996 to present in 63 countries of Asia, Europe, Middle East, and Africa and H7N7 in the Netherlands, Belgium, and Germany [95]. Vaccination of poultry had been used in four epizootics to control the outbreaks because of inadequate control with traditional means. These include: Mexico in1994–1995 for H5N1 epizootic, Pakistan in 1995–2004 for H7N3 epizootics, Asia/Africa/Europe from 1996 to present to control the ongoing H5N1 epizootics, and North Korea in 2005 for a H7N7 epizootic [95]. From 2002 to 2010, over 13 billion doses of avian influenza vaccines had been used in poultry, inactivated whole virus vaccines in 95.5% and live vectored vaccines in 4.5% [96]. Most vaccines, 91.9%, were used to control HPAIVs, and only 8.1% were used for LPAIVs, H5 and H7 strains. Over 99% of vaccines used for HPAIV H5N1 were for the four enzootic countries: China, including Hong Kong [91%], Egypt [4.7%], Indonesia [2.3%], and Vietnam [1.4%] where vaccination programs have been routine and nationwide to all poultry [96]. Bangladesh and Eastern India have enzootic H5N1 HPAIV but have not used vaccination in their control programs.

Overall poultry vaccination has been found to be beneficial in reducing clinical disease and mortality in chickens and ducks and lessens the risk of human infection and economically appears to be cost-effective [95]. A recent meta-analysis on the efficacy of avian influenza vaccines [against H5N1 or H5N2 viruses] reported efficacy on four outcomes for homologous inactivated vaccines: protection against mortality 92%, morbidity 94%, reduction in respiratory virus 54%, and reduction in virus excretion from the cloaca 88% and somewhat less for inactivated heterologous vaccines [97]. Field outbreaks have occurred in vaccinating countries mainly because of inadequate vaccine coverage of susceptible fowls or only after a single dose, but vaccine failures have occurred following antigenic drift in the four main vaccinating countries [95]. Influenza vaccines for swine and poultry are primarily conventional inactivated preparations, but there are novel vaccines in the field and under development ranging from nuclei acid-based vaccines, replicon particles, subunits and virus-like particles, vectored vaccines, and live attenuated vaccines [98].

Development of human vaccines for avian influenza H5N1 and H7N9 has gained interest in recent years because of their perceived potential to cause pandemic

outbreaks. However, vaccines in development against these viruses have been weakly immunogenic. Two recent studies using adjuvanted vaccines showed promise for the development of effective avian influenza vaccines in humans, but likely with multiple doses. Various doses of a monovalent inactivated surface antigen H5N1 influenza vaccine, with or without the MF59 adjuvant, were tested in 565 vaccine-naïve adults and 72 subjects who were vaccinated a year before with an older Vietnam vaccine [99]. Low-dose adjuvanted vaccine was more effective than high-dose unadjuvanted vaccine after two doses given 28 days apart in generating effective hemagglutinin inhibition titers of >1:40, but local and systemic reactions were higher. A single dose of vaccine with or without adjuvant had a boosting effect on subjects vaccinated a year before in 21–50% [99]. Another recent study assessed the immune response to a split virus inactivated monovalent avian influenza H7N9 vaccine with or without the same adjuvant [100]. The vaccine was given twice 28 days apart in various doses in 700 participants in seven groups. At the lowest dose with adjuvant seroconversion occurred in 59% of subjects, but there was no data on antibody titer after 42 days. There was no serious reaction to the vaccine, but subjects given adjuvants had greater local reaction than those without. An unexpected finding was the attenuated response in participants who received recent seasonal influenza vaccine, similar to the response in older subjects. Another approach is the administration of priming, live attenuated influenza vaccine [pLAIV] against influenza A [H7N9], followed 12 weeks after with the candidate pandemic inactivated unadjuvanted influenza vaccine [pIIV]. A study in healthy young and older [18–49 years and 50–70 years] volunteers demonstrated strong immune memory with subsequent antigenic challenge [101].

Further research is needed to develop more effective single dose avian influenza vaccines, as any multiple dose vaccination program for large-scale use in an emergency setting, such as an impending pandemic, would be an obstacle to achieve adequate protection of the global population. Furthermore, it is unknown whether or not a hemagglutinin inhibition or neutralizing antibody titer of >1:40 would be effective to prevent human infection with the avian influenza viruses [102]. A model on the efficacy of vaccination program in the setting of an emerging influenza pandemic found that timeliness of vaccine production and administration would have the greatest impact even for an effective vaccine [103]. Starting a vaccination program in the US 16 weeks before the onset of a major epidemic, with an estimated 30% clinical attack rate and production of 30 million doses per week, would result in 38% reduction in hospitalizations and deaths. Delaying the start of the vaccination program to the same week of an outbreak decreases the reductions in severe morbidity and mortality to only 18% [103]. In addition, administering only 10 million doses per week would result in lower benefit to 21% with an early program and to 6% when delayed.

3.6.2   Treatment of Zoonotic Influenza

Neuraminidase inhibitors [NAIs], oseltamivir, peramivir, and zanamivir, are the only licensed agents for treatment of circulating influenza and for zoonotic strains. There is debate about their effectiveness in uncomplicated influenza in healthy

people, and randomized studies only showed a reduction of symptoms by 1 day [104–106]. However, observational case-control studies indicate that early administration [within 48 h of symptoms] of oseltamivir can reduce the morbidity and mortality of severe infections in hospitalized patients with influenza [107, 108]. A recent meta-analysis of observational studies on the benefit of NAIs during the swine H1N1 influenza pandemic concluded that early treatment was effective in reducing the mortality and severe outcomes compared to late or no treatment, odds ratios 0.35 and 0.41, respectively [108].

In a study from CDC, a spread-sheet model was used to calculate the potential benefit of NAIs in a severe H7N9 avian influenza outbreak in the US. It was estimated that the demands could be met with the current supply available. Early treatment with these antivirals could prevent 5200–248,000 deaths and 4800–504,000 hospitalizations, but there still would be large number of deaths [25,000–425,000] and hospitalization [500,000–3,700,000] [109]. Although there is no good clinical evidence on the efficacy of NAIs against the avian influenza strains H5N1 and H7N9, these viruses have been found to be susceptible in vitro. Data collected on patients with severe H5N1 avian influenza infection treated with oseltamivir showed limited efficacy with mortality rate still around 50% [110, 111]. Furthermore, during the first wave of human infection with H7N9, six patients treated with NAI had developed resistant variants, and three died [112]. A NA-R292 K mutation that confers broad-spectrum NAIs resistance after treatment was detected. Prospective data on all patients infected with H7N9 and treated with a NAI should be collected and screened for mutations to determine the frequency of antiviral resistance.

3.7 Future Directions

There are several issues and exigencies that should be addressed in order to prepare adequately for a severe avian influenza pandemic. These include: (1) measures to prevent and reduce cross-species transmission, (2) means of improving vaccine efficacy and rapid production of large supplies, (3) development of more effective novel antiviral agents, and (4) improvement in the coordinated global emergency response to an emerging influenza pandemic. In order to prevent cross-species transmission of zoonotic influenza, further research is needed to elucidate the mechanisms and mutations necessary for this to occur. The pragmatic approach taken in Hong Kong when faced by an outbreak of zoonotic influenza does not provide a permanent solution. Modern hygienic facilities for animal husbandry are needed in developing countries, and plans to fade out live animal markets and local backyard farms need to be gradually implemented.

Use of animal vaccines for epizootic and enzootic influenza viruses should be expanded to more countries and greater proportion of animals for both HPAIV and LPAIV. However, the constant antigenic shift and drift of the influenza viruses poses significant challenges to develop and mass produce new vaccines every year for both humans and animals. The ideal solution would be the development of a univer-

sal vaccine that covers all strains with development of antibodies to non-variable and highly immunogenic epitopes. Current vaccines are based on administration of purified immunodominant envelope glycoproteins [HA and NA], which are highly variable and drift under immune pressure. In addition most neutralizing antibodies are directed against the strain-specific globular head of HA [98]. Development of more broadly neutralizing and cross-reactive antibodies with novel vaccines should be feasible, against receptor-binding site on HA1 subunit and the fusion machinery of the HA2 subunit [113]. The most pressing need, however, as demonstrated by the 2009 pandemic, is to produce sufficient influenza vaccines for a novel strain in a short period of time for an impending epidemic. The traditional influenza vaccines are produced in embryonated chicken eggs cultures and require a lead time of 6 months for production of a new vaccine in sufficient quantities. The cell culture systems can produce the vaccines more rapidly, but the amount of antigens produced is less than with eggs. Several approaches will be needed to overcome these limitations such as licensing more companies to make influenza vaccines and encourage rapidly developing countries [China, India, and Brazil] to produce influenza vaccines for their own and the regional needs and more reliance on cell culture methods. Also lower doses of vaccines can be given by intradermal injection and may be just as or more effective than intramuscular administration and hence provide a greater supply of vaccines. The safety and efficacy of live attenuated avian influenza vaccines in humans should be assessed. Currently live attenuated intranasal seasonal influenza vaccines are available for children and young adults. The present evidence indicates that these vaccines are safe and more immunogenic than conventional inactivated vaccines and can produce longer-lasting antibodies. The fear that the live virus in these vaccines could mutate to become more virulent and cause influenza disease has not been found to date.

Development of new classes of antivirals is important for preparing for future influenza pandemics, especially for treatment of severe cases and in high-risk subjects. Peramivir is now available in the US for intravenous therapy, but this agent has the disadvantages of the NAI-class, questionable efficacy for avian influenza and increasing reports of drug resistance. A promising drug is a new broad-spectrum antiviral agent favipiravir [Toyama Chemical], which is approved for emergency treatment of influenza virus in Japan [114]. Potential targets for development of new agents include polymerase inhibitors [i.e., T-705] and attachment inhibitors [i.e., DAS-181] [115]. Patients with acute lung injury [ARDS] secondary to avian influenza have a high mortality, and antihuman anticomplement C5a antibody may be an effective novel treatment that should be tested in humans. A recent study in monkeys with avian H7N9 influenza virus infection demonstrated that anti-C5a significantly reduced the systemic inflammatory response and the viral load in the lungs [116]. Intravenous immunoglobulin [IVIG] contains broadly cross-reactive antibody-dependent cellular cytotoxicity against heterologous influenza strains [including the swine H1N1 strain] and has been proposed for use in critically ill patients with influenza [117]. However, for patients infected with an emerging avian influenza virus, the source of the IVIG would preferably be derived from previously exposed subjects with evidence of existing antibodies to the avian virus.

3.8   Conclusion

Zoonotic influenza A viruses, particularly pathogenic avian viruses, will continue to pose a serious global public health risk for the foreseeable future. Better understanding of mechanisms of cross-species transmission is direly needed in order to prevent these happenings. Although current data shows no impending risk of a major avian or zoonotic influenza major outbreak, continued surveillance and vigilance should be maintained. Presently the two avian viruses of public health interest [H5N1 and H7N9] have not mutated to become more easily transmissible from birds to humans or humans to humans. However, of some concern is a recent report from China of a probable nosocomial transmission of avian influenza A [H7N9]. A 57-year-old male, with a history of chronic obstructive lung disease, developed acute influenza A after sharing the same hospital ward with an index patient for 5 days. The index patient became ill 7 days after visiting a poultry market, but the secondary case had no bird contact. Both patients died and the influenza A [H7N9] isolated from both patients genome sequences were nearly identical and genetically similar to the virus isolated from the live poultry market [118]. Thus, continuous monitoring for further cases of human-to-human transmission for this avian influenza virus is critical.

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