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Avian/Bird flu: A review: H5N1 outbreaks in Nepal

Dhiraj Shrestha[1], Balkrishna Bhattachan[2], Hiramani Parajuli[3], and Sujata Shrestha[4] *
[1]Department of Microbiology, Shi-Gan International College of Science and Technology, Kathmandu, Nepal.
[2]Biotechnology Society Nepal, BSN Bulletin, Bhaktapur, Nepal.
[3]Department of Microbiology, Tri-Chandra Multiple College, Kathmandu, Nepal.
[4]Department of Laboratory, Central Jail Hospital, Kathmandu, Nepal.
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Abstract

Avian/Bird flu is a viral disease of birds, caused by avian influenza virus (AIV). A highly pathogenic avian influenza (HPAI) H5N1 has breached the barrier of species to humans and other animals escalating the pandemic threat. If the H5N1 evolves to a human-to-human transmissible virus retaining its pathogenicity, it can trigger an influenza pandemic. H5N1 has a mortality rate of about 60%, varying with strains. Meaningful antigenic alteration in hemagglutinin (HA) and/or neuraminidase (NA) results in recurring pandemics. The HPAI H5N1 subtype alone has outreached more than 77 nations around the world since the first human case and death was reported in 1997. Wild and migratory birds are the AIV reservoirs. Poultry is primarily impacted by incidents and outbreaks of the disease. A wide range of serological and molecular methods have substantially aided in the identification of bird flu in humans. Candidate vaccines have been developed, yet are not ready for widespread use. Oseltamivir (brand name: Tamiflu) is the preferred drug for the management of human Influenza-like illness (ILI). Surveillance, mass awareness, and pandemic preparedness abiding WHO recommendations are of paramount importance for the prevention of bird flu outbreaks.

Keywords: Avian Influenza Virus (AIV), Avian Flu, Bird Flu, H5N1, Nepal

Corresponding author, email: ssuju.046@gmail.com

Introduction

The word 'influenza' has Italian origin meaning 'influence'; from the belief that epidemics were due to the influence of the stars [1]. It became a term for a particular disease after the 1743 epidemic. But, the virus was isolated from a human only in 1933 [2, 3]. Avian influenza virus (AIV), better known as bird flu, is a type-A influenza virus of the Orthomyxoviridae family. Theoretically, owing to the combination of antigens, hemagglutinin (HA) and neuraminidase (NA), type-A influenza comprises thousands of distinct antigenic subtypes [4]. To date, 18 subtypes of HA and 11 subtypes of NA have been identified, while two extra subtypes of HA and NA have been identified in bats [5, 6]. World Organisation for Animal Health (OIE) defines AIV as "an infection of poultry by any influenza A virus, including by subtypes H5 and H7" [7]. OIE requires notification for all low-pathogenic avian influenza (LPAI) virus outbreaks, i.e. H7 and H5 subtypes as they can mutate into highly pathogenic avian influenza (HPAI) viruses, as documented in some poultry outbreaks. Non-H5 and non-H7 LPAI are not deemed notifiable [8]. In certain poultry, such as ducks, some HPAI virus (e.g., H5N1) have been shown to cause no illness. HPAI has been associated with AIV subtypes H5 and H7, including the viruses H5N1, H7N7, and H7N3. Human infections have varied from moderate (H7N3, H7N7) to severe and lethal (H5N1) infections [9].

The HPAI H5N1 virus has raised concerns around the world as it threatens poultry, especially chickens; also it has shown the potential to pass from poultry to humans and caused severe infection and death [10]. The first reported human infection of H5N1 occurred in 1997 in Hong Kong [11]. The virus has been endemic in several countries after the re-emergence of H5N1 in Asia, Africa, the Pacific region, Europe, and the Middle East in 2003, and continues to cause poultry outbreaks [12]. In Dhaka, the presence of the H5N1 virus was confirmed in March 2007 [13]. The first case of AIV was identified at a remote non-commercial poultry farm in Kakarvitta, Eastern Nepal, on January 16, 2009 in Nepal [14, 15]. More than 256 H5N1 outbreaks has been witnessed in poultry since 2009 [16].

Incidence and Prevalence

Climate change can bring a quick ecosystem shift which alters the evolution and ecology of infectious diseases including AIV. This ecosystem shifts had played a

cumulative effect in the outbreak of the influenza pandemic in the past century [17]. Furthermore, studies show an increase in temperature of freshwater in temperate and Arctic regions had a persisting impact altering the suitable habitat variation in prevalence rate and viral spreading [18]. Several studies have posited agricultural system, poultry trade, availability of water temperature, salinity as factors resulting in an alteration of AIV ecology [19, 20]. Studies have shown that the occurrence of new AIVs is supported by either point mutation, partial genes recombination, or genetic re-assortment of the whole genome. Most new strains of AIV evolved due to point mutations while genetic re-assortment performs a key task in the genesis of H5N1 and H7N9 strains [21]. The pathogenicity of AIV is associated with efficient virus replication. Host immune responses and the genetic markers are determinants for efficient virus replication [22]. The occurrence of human influenza A (H5N1) often parallels with massive outbreaks of avian H5N1 influenza A, while avian outbreaks in 2004 and 2005 have seldom led to human infection [23]. Different types of studies based on geographical distribution and outbreak of AIVs revealed that prevalence is related to the migratory route and stages of the itinerary of migrating birds [24, 25]. Studies have concluded that Asia is the prevalent continent for the avian influenza virus due to a unique ecosystem of various lakes, wetlands, creeks, and rivers that constitute migratory bird wintering areas [26]. Due to consequence and the risk represented to human health, AIV is of great concern and are widely studied in recent times. Wild birds of genera Antidae (ducks, geese, and swans) and Laridae (gulls, terns, and kittiwakes) have been in focus for decoding the epidemiological links between hosts and transmissions of AIV [27].

Etiology

The pandemic influenza virus has its origins in avian influenza [28]. Its genomic material is composed of eight segmented negative strand RNAs. The influenza A virus shows external spikes when examined with an electron microscope. Various techniques can distinguish two kinds of spikes. The HA molecules made up one type and the NA molecules the other. There are roughly 5 times as many HA spikes as NA spikes [29] (Figure 1). The 18 different subtypes of HA and 11 different subtypes of NA exist allowing for 198 potential different viral strains. As of 2019, only 131 subtypes have been detected in nature [30].

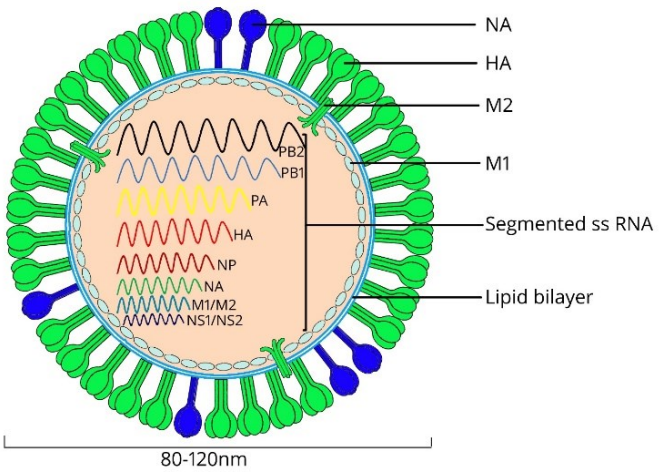


Figure 1. Schematic diagram of the Influenza A virus with the virus components. Note: NA=neuraminidase, HA=hemagglutinin, M1=matrix protein-1, M2=matrix protein-2, ss RNA=single stranded RNA, PB2=polymerase basic-2, PB1=polymerase basic-1, PA=polymerase acidic, NP=nucleoprotein, NS1=non-structural protein-1, NS2=non-structural protein-2.

Genomic variation

Genetic analyses have indicated that since 1997 the H5N1 virus has evolved into multiple genotypes [31]. Whether the genetic modifications in the HA protein are the result of immune escape or are related to host adaptation is not clear [32]. Hence, the best H5N1 epitopes is difficult to predict to target with vaccines without understanding the antigenicity of emerging strains. As H5N1 viruses have expanded their geographical and host ranges, it has become increasingly important to determine acquired features that permit successful human-to-human transmission. The transmission of H5N1 viruses to humans has been ineffective, arising either by direct interaction with infected poultry or through ingestion of undercooked meat or blood of infected birds [33]. In influenza A viruses, antigenic drift is a continuous mechanism where point mutations occurs during replication of the viral genome. This mechanism is apparent in all the influenza A virus gene products; however, it is most pronounced in the HA and NA glycoproteins' antibody-binding sites. These mutations cannot be repaired because of the lack of a proofreading mechanism, and the resulting aggregation of variations in the amino acid sequence transforms these antigenic sites in such a way that they are less detectable by the host antibody response. Thus, these virus strains are naturally selected as host antibodies no longer no longer neutralized them, and they lead to increased viral fitness [34, 35].

Antigenic Variation

Both the HA and the NA undergo antigenic variation [36]. Antigenic shift is a major change in antigenicity, while antigenic drift is a minor change sufficient to spark a new outbreak. In the human population, pandemics happened in 1957 (Asian influenza) and 1968 (Hong Kong influenza); both are associated with the presence of antigenically transformed HA molecules. The HA antigenic variation is known to be more important quantitatively than that of the NA. The human pandemic virus of 1918 (Spanish influenza) was the common ancestor of human and classical swine H1N1 influenza virus [37, 38].

Epidemiology

Avian flu or bird flu was initially known as Fowl Plague, and later Fowl Plague virus was discovered as an influenza virus. The outbreak of avian flu was first documented in 1878 in Italy. Later on, in 1924 and 1929 two massive outbreaks in poultry were recorded in the United States [39]. Since the first reported human case and death by the influenza virus in 1997 in Hong Kong, the eruption of the disease has been documented from wild and domestic birds including humans. HPAI H5N1 alone has been reported from over 77 countries [40, 41]. Till the date, a total of 860 human cases have been reported since 2003 with more than 50% deaths by H5N1[42]. Since 2013, 1,568 human cases and 616 deaths were reported worldwide attributed to the novel H7N9 [43]. Geographically the disease is globally prevalent dominating the Asia continent showing the higher outbreaks in China, Vietnam, India, Taiwan, Israel, Japan, and South Korea [26].

Host range, transmission, and spread

AIV is found distributed in a wide variety of hosts. Predominantly free-flying water birds, like geese, ducks, shorebirds, and gulls, are major reservoirs of AIVs. Besides this, AIV can infect both wild and domestic birds like chicken, turkeys, partridges, pheasants, quails, pigeons, and ostriches [44]. However, the AIVs primarily associated with transmission in chickens but not in ducks were found to be adapted to ducks by acquiring genes from duck influenza viruses [45]. AIV is essentially a disease of birds, but evidence has shown that the infection can be transmitted in cats, dogs, eagles, ferrets, hamsters, horses, humans, macaques, marine mammals, mice, minks, pigs, and tigers; but the zoonotic infection had been only reported in China [46]. The transmission and spread of disease were primarily related to poultry contact, yet human-to-human transmission is also possible [47]. Moreover, the outbreak of avian flu is also associated with the vicinity of water source and 97.5% of reported cases showed the proximity of water source [26].

Virus replication

The influenza A virus genome consists of segmented RNA encoding 10 proteins [48]. These 10 viral proteins are necessary for a successful infection cycle within immuno-competent hosts. On the surface of the host cells, the HA protein binds to sialic acid, enabling the virus to enter [49]. The host cell endocytoses the virus particles. The endosome maturation lowers the pH triggering a HA conformational change. This results in the membrane fusion of the endosome and the virion. The viral matrix protein-2 (M2) acts as an ion channel that further lowers the pH of the virus particle. This aids in the dissociation of the matrix protein-1 (M1). The virus ribonucleoproteins (vRNPs), including polymerase basic-1 (PB1), polymerase acidic (PA), and polymerase basic-2 (PB2), are released into the cytosol [50]. For viral replication and transcription, the vRNPs are then moved into the host cell nucleus. The nuclear export protein (NEP) and M1 traffic out freshly produced vRNPs into the cytoplasm and then to the plasma membrane after replication, transcription, and protein synthesis. Several viral proteins, including M1 and M2, contribute to budding. Finally, in both the viral and host cell membrane, NA removes sialic acid from glycoproteins. This stops HA and host cells from interacting, releasing new infective virus particles [1]. Nonstructural protein 1 (NS1) counteracts the innate host-cell defense within the infected cell, including interferon, for efficient virus replication [51]. New proteins generated by co-transcription or co-translation with the host are being continuously identified [52].

Pathogenesis

Virulence factors comprise the highly cleavable HA that several cellular proteases can activate, a specific substitution in the polymerase basic protein-2 enhances viral replication [53]. Non-structural protein-1 substitution confers improved resistance to interferons and tumor necrosis factor-alpha inhibition [54]. The H5N1 viruses potently induce proinflammatory cytokines in macrophages, the most notable being tumor necrosis factor-alpha, which may contribute to the unusual severity in humans [55]. With antigenicity change [56], constellation of internal gene, expanded range of avian species [57], enhanced pathogenicity [58], and increased environmental stability, these viruses keep

evolving. Despite extensive exposure to infected poultry, comparatively low frequencies of H5N1 infection in humans suggest that the species barrier to acquisition of this avian virus is important [33].

Disease in birds

Avian flu has been reported in many wild and domestic birds. Chickens, ducks, swans, gooses, quails, crows, storks, etc. have been known to have been infected with AIV. It is understood that H5N1 infections show distinct clinical presentations in chickens and ducks. The infection is characterized by clinical signs and high mortality in chickens, whereas the infection is generally asymptomatic in ducks. This leads to an underestimation of the prevalence of the disease [59]. Infected ducks can thus help to sustain and spread the virus "silently" to other vulnerable hosts [60]. The H5N1 infection in ducks has been considered a threat to the poultry flock and public health due to its silent nature [57]. Thus, monitoring and surveillance based on ducks is substantial for AIV control [61]. Since ducks may become infected and co-infected with multiple AIVs, re-assortment of the viral genes is likely [62]. Spatial evidence on HPAI outbreaks in Southeast Asia has shown that scavenging ducks lead to HPAI outbreaks in domestic poultry [63]. Evidence shows that ducks play a major role in the transmission of HPAI since they do not exhibit the disease symptoms.

Characterization of virus

In 1996, the HPAI H5N1 virus was found in Gundong province, China which was thought to have originated from the H5 virus in migratory birds through both drift and shift mutation mechanisms [64]. Since then the influenza virus in human cases has evolved and is classified in a different list based on hemagglutinin phylogeny known as a clade. This list of the clade is updated for continuous change [65]. There are 13 major clades in the list distinguished by the hemagglutinin gene of the H5N1 representative subtype of HPAI. These clades are determined by sequencing and measuring the average pairwise nucleotide distance (APD) [66]. These clades were further split by measuring the within-clade average pairwise distance to determine the sub-clade resulting in more than 32 clades or sub-clades [67].

Clade 0 comprises viruses that were first identified to cause human infections in Gundong province, China 1996 (A/goose/Guandong/1/96 linage). In the early phase of epidemic (2004-2005), clade 1 viruses predominated in Vietnam, Thailand, and Cambodia, and clade 2.1 viruses are endemic in Indonesia. The clade 2.1.3.2b was found to be restricted to a certain geographical area i.e. Java, Indonesia [68]. A major outbreak of H5N1 disease in migratory birds has been associated with clade 2.2 and the virus is distinct from Z genotype virus and was first detected in Russia, Kazakhstan and later spread, causing avian disease in Africa, Central Asia, Europe, the Middle East, South Asia, and human disease in western Africa, Asia, and the Middle East resulting pandemic in 77 countries [69]. In southern China, clade 2.3 has been dominant and has also been reported in adjacent countries. Later on, clade 2.3.2.1c was detected in Canada and Austria. Clade 7 was restricted to China and Vietnam in the year 2006-2009 [70].

The pattern of viral replication

The characterization of virologic course of AIV H5N1 is yet to be done. Many studies reported prolonged viral replication. AIV can be detected in nasopharyngeal or lower respiratory sites ranging from 1 to 16 days. Nasopharyngeal replication has been lower in humans as compared to lower respiratory tract replication [71]. AIV has also been documented to replicate in the gastrointestinal tract [72, 73]. The invasive infection has been documented in humans [72]. The HA cleavage site's polybasic amino acid sequence is linked with visceral dissemination in birds [33].

Host immune response (Innate type)

Despite widespread exposure to infected poultry, the comparatively low disease frequencies in humans illustrate the species barrier of AIV [33]. H5N1's ability to infect birds or humans tends to be partially determined by the HA binding specificity. Generally, HAs of human strains of influenza virus preferentially bind sialic acids bound to the terminal galactose of the oligosaccharides on the cell surface by α 2,6 linkage (SA α 2,6), which is abundant in human respiratory epithelia [74]. The HA of avian strains, on the other hand, binds preferentially to α 2,3-linked sialic acids (SA α 2,3). These linkages are common in the avian intestinal tract [75]. For host cell infection and the dissemination and virulence of influenza viruses,

For host cell infection and the dissemination and virulence of influenza viruses, HA interaction with sialylated glycans on the cell surface is important [76]. Mutations altering the specificity of the receptor binding of avian viruses may be essential for avian to human crossover, and for enabling direct human-to-human transmission [77]. Pathogenesis of disease is contributed by the innate immune response [33]. Elevated blood

levels of interleukin-6, interleukin-8, interleukin-1 *b*, soluble interleukin-2 receptor, tumor necrosis factor (TNF), TNF-*a*, interferon-*g*, monokines induced by chemokines interferon-inducible protein 10, interferon-*g*, and monocyte chemoattractant protein 1 were observed in the fatal cases [71, 78]. Such responses can be attributed to the sepsis syndrome, acute respiratory distress syndrome (ARDS), and multiorgan failure. Corticosteroids are used to minimize such responses. Individuals developing specific humoral immune responses can survive [33].

Transmission

Transmission of AIV occurs through direct contact via the inhalation of droplets and droplet nuclei. Transmission also occurs perhaps through indirect (fomite) contact and self-inoculation onto the upper respiratory tract or conjunctival mucosa [79]. The data is consistent with bird-to-human, potentially environmental-to-human, and limited human-to-human transmission to date. Animal to human transmission is mainly attributed to exposure to live ill poultry and butchering of birds [80]. In addition to plucking and preparing infected birds, handling of fighting cocks, handling poultry notably asymptomatic infected ducks, and eating of undercooked poultry or duck's blood has also been proposed for transmission of AIV. Also, feeding tigers and leopards in zoos with raw, infected chickens has been proposed for transmission of AIV [81]. Human-to-human transmission has also been manifested in recent years. But no case of human-to-human transmission through aerosols of small particles or through social contact has been reported. Still, severe illness has been reported in clinicians exposed to an infected patient. The survival of H5N1 in the environment outlines the possible mode of transmission. Direct intranasal or conjunctival inoculation, and oral intake of contaminated water during swimming is the possible mode of transmission. Also, contamination of hand and subsequent self-inoculation is the possible mode of transmission. The use of untreated poultry feces as fertilizer has increased the risk of environmental transmission [82].

Clinical features

Incubation:

The incubation period of H5N1 infection is 2–4 days after exposure to infected, sick, or dead poultry [83]. But incubation time can be prolonged up to 8 days [33, 84]. The degree of virus shedding during this time is still unknown [85-87].

Initial symptoms

The most common and initial symptoms that appear after H5N1 infection is respiratory distress. Milder cases show uncomplicated flu-like illness [88]. Pneumonia is often of viral origin with no bacterial superinfection, but may differ from cases to cases [78]. Crackles on examination of the chest are also seen [89]. Other frequently occurring symptoms include a high fever with >38°C, sore throat, cough, shortness of breath, rhinorrhoea, etc. [33]. Some cases suffer from diarrhea, vomiting, and abdominal pain apart from typical clinical manifestation [90]. Conjunctivitis or upper respiratory infections are not common [87]. Further complication includes multi-organ failure like renal disorder, cardiac malfunction, and pulmonary hemorrhage. Reye's syndrome, pneumothorax, ventilator-associated pneumonia, and ARDS are also seen in some cases [33]. Though central nervous system involvement is rare there are cases where convulsion and progressive coma are reported leading to the death if not treated on time [90, 91]. Two patients in Vietnam were detected with encephalitis only [92].

Clinical course

A patient infected with H5N1 has a rapid clinical course, developing progressive lower respiratory tract disease and viral pneumonia. Mechanical ventilation may be needed depending upon the severity of the case [33]. ARDS following the multi-organ failure develops in 68% of patients within six days of disease onset [91]. The average duration from onset of disease to hospital admission is four days and from onset to death in critical cases is nine days [93]. Few cases of seropositive patients but without typical manifestation are also reported [94].

Mortality/Morbidity

The mortality rate of H5N1 is significantly high and is at an alarming rate accounting for about 60% [42]. But the rate may increase up to 73% in the youth of the age group 10-19 years followed by 18% in the age group 50 years [95]. The mortality rate was even 90% in severe cases [96]. Most of the death cases are frequent with late admission [97, 98].

Diagnosis

Specimen for upper respiratory tract infections

Nasal swab/nasal wash

Polyester or Dacron swab is recommended with an aluminum or plastic shaft. The dry swab is inserted in the internal nares below the inferior turbinate. It is then allowed to absorb secretion for some time and is rotated

around the area before the withdrawal. The tip is then broken and dipped in viral transport media (VTM) vial. For nasal wash, physiological saline is used [99]. The patient is advised to close the pharynx by saying the letter "K" and the saline is introduced in nostrils one at a time with the head tilted backward and later the saline is collected in a cup or dish by tilting the head forward. The obtained wash is then mixed with the VTM [100].

Throat swabs

A throat swab is considered to be the high yielding specimen for upper respiratory tract infections and should be obtained within three days after symptoms appeared for the best result [100]. By using the tongue depressor or blade, polyester or Dacron swab is gently rubbed several times in the posterior pharynx. The tip is then broken and dipped in a VTM vial [99].

Nasopharyngeal secretions

A catheter with a mucus trap is inserted in nostrils. By use of a vacuum, the catheter is withdrawn with a rotating motion. After that, the catheter is flushed with a transport medium in a 1:2 ratio [99].

Specimen for lower respiratory tract infections

An endotracheal aspirate or bronchoalveolar lavage is the best specimen for lower respiratory tract illness. For increasing the potential of viral isolation, multiple specimens can be taken from multiple respiratory sites for at least two consecutive days [72].

Blood samples

Both acute and convalescent serum samples are recommended if feasible. Within the first 3-5 days after the onset of symptoms, acute serum samples should be taken. Convalescent serum samples collected after 3-4 weeks would be of great use if collected in conjunction with acute samples [99, 101].

Other specimens

It may be either plasma or rectal swab in diarrhea for the detection of viral. Spinal fluid/ tap is also preferred in the case of meningitis [100]. Autopsy specimens along with para-mortem biopsies are also recommended [28].

Transportation and storage of specimens

VTM contains balanced salt solutions, a protein stabilizer, bovine albumin, and a spectrum of antibiotics to minimize bacterial and fungal growth. Non-phosphate based VTM is selected if the specimen is only suggested for the PCR test. The collected specimen is placed in VTM and refrigerated at 4°C or with refrigerated packs for transportation to the designated laboratories [100].

The clinical specimens should be well labeled and sent to the laboratory as soon as possible. If specimens cannot be processed, then it should be either stored at 4°C or frozen at ≤-70°C. Samples should not be repeatedly freezed0 and thawed [102]. In the case of viral antigen detection by immunofluorescence staining, specimen processing should be done as soon as possible not more than 1-2 hours after collection [83]. A guide for field operations is recommended regarding the collection, storage, processing, and even transportation of samples for H5N1 detection [100].

Laboratory diagnosis

Since most of the respiratory infections show similar manifestations, H5N1 can only be diagnosed in account with endemic areas and contact with dead or infected poultry or with confirmed cases.

Direct detection of virus or viral antigen

Virus isolation or reverse transcriptase PCR (RT-PCR)

Virus isolation is considered to be the gold standard method and can be carried out in the laboratory either by inoculation of embryonated eggs or by using cell lines like Mardin-Darby Canine Kidney (MDCK). The obtained viral isolate can be later used for the study of pathogenicity, antiviral resistance, and DNA sequencing and analysis. But culture needs a biosafety level 3 (BSL-3) which is not available at ease. So, RT-PCR is used as the first diagnostic tool in contrast to culture [85, 86].

Real-time PCR or quantitative PCR (qPCR)

qPCR methods are preferred to conventional RT-PCR. There is a panel of qPCR assays covering even the specific detection of different NA genes and HA subtypes like H5, H3, and H1. H5 and N1 specific primers are used as they exclude false-negative results due to mutation in genes [72, 103]. Loop-mediated isothermal amplification (LAMP) tests are no longer in use [104, 105].

Serology

The Antigen detection

For the detection of viral antigens by using specific monoclonal antibodies against H5 and N1 direct immunofluorescence and enzyme immunoassay (EIA) are commonly used. The EIA method is considered simple, convenient, sensitive, and even applicable as Point of Care testing (POCT). The sensitivity of EIA is 1,000-folds low compared to viral isolation. Thus, subtype-specific diagnostic methods like RT-PCR should be carried out simultaneously [106].

Antibody detection

Since seroconversion is delayed and needs paired sera, antibody detection provides a retrospective scenario of infection. It can be done by detecting a four-fold rise

while comparing acute and convalescent sera. Hemagglutination Inhibition (HI) assay is conventional yet the preferred method. But HI has low sensitivity for the detection of subtype-specific antibodies [107-109]. The use of horse erythrocytes while detecting antibodies against H5N1 shows effective results [110]. One of the studies done in 1997 during the Hong Kong outbreak reported similar results and reported that the micro-neutralization test is more effective, reliable, and sensitive as compared to HI [111]. Western blot analysis with recombinant H5 is also done for the conformation [112-114].

Hematological Profile

It includes leukopenia, relative lymphopenia, and even thrombocytopenia. Disseminated intravascular coagulation occurs but is rare [33, 84].

Biochemical parameters

Levels of liver enzymes are elevated, along with lactate dehydrogenase and creatinine kinase, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) [85, 86].

Others

Blood culture, sputum culture, and CSF analysis are carried out in case of serious complications. Chest X-ray reveals effusions, multifocal consolidation, lymphadenopathy, and even shows diffuse ground-glass appearance as in the case of ARDS [71, 78].

Differential diagnosis

Diagnosis of AIV should be differentiated with atypical pneumonia, community-acquired pneumonia (CAP), corona-virus disease 2019 (COVID-19), endemic respiratory infections, hantavirus pulmonary syndrome, middle east respiratory syndrome (MERS), pediatric pneumococcal infections, pneumococcal infections (*Streptococcus pneumoniae*), respiratory syncytial virus infection, seasonal influenza, and severe acute respiratory syndrome (SARS) [83, 94, 115].

Definition of exposures to poultry and humans

Human exposure definition differs from the environments and the situation (**Table 1**).

Table 1. Definition of exposures to poultry and humans [116].

Exposure to live poultry in occupations
Poultry-related exposure at workplace (e.g., persons engaged with poultry raising, trafficking, selling, and quarantine) within 2 weeks of the start of symptoms
Exposure to poultry at live bird markets
Visiting a live poultry or bird wholesale or retail market within 2 weeks before the start of symptoms
Exposure to sick or dead poultry
Close or direct physical contact with infected or dead poultry or poultry products (e.g., meat) or feces within 2 weeks before the start of symptoms
Exposure to backyard poultry
Close or direct contact with poultry raised in the backyard within 2 weeks before the start of symptoms
Any exposure to poultry
Close or direct or indirect contact to healthy, or sick, or dead poultry (including birds-e.g., chickens, ducks, geese, pet birds, pigeons) in live bird markets, or backyards, or farms, or neighborhoods, or consumption of improperly processed poultry products
Exposure through patient contact
A patient with a history of close contact within 2 weeks before the start of symptoms with a person with a confirmed or suspected influenza H5N1 virus infection (at any time from the day before the onset of symptom to death, or during the period during which the patient was hospitalized)

Case definitions [117, 118]

For prompt diagnosis and treatment, WHO has defined H5N1 infection as:

Person under investigation

A person who is decided to be investigated for possible H5N1 infection by assigned public health authorities

Suspected H5N1 case

A person with fever (>38°C) exhibiting unexplained acute lower respiratory distress along with cough, shortness of breath, or dyspnoea.

AND

In the 7 days prior to symptom onset, one or more of the following exposures:

a) Contact with a person within 1-meter distance during caring, speaking, or touching whether s/he is a suspected, probable, or confirmed H5N1 case.
b) Exposure either to poultry in an area suspected or confirmed with H5N1 infections not more than a month or wild animals during handling, slaughtering, de-feathering, etc. and even contact with the contaminated feces.
c) Intake of poultry products raw or undercooked in an area where animal or human H5N1 infections is reported or confirmed in the past month.
d) Close contact with an animal infected with confirmed H5N1 other than poultry or wild birds (e.g. cat or pig)
e) Handling animal or human samples in a laboratory or other facility suspected of having the H5N1 virus.

Probable H5N1 case (notify WHO)

Probable definition 1:

A person who satisfies the conditions for a suspected case

AND

Additional one of the following criteria:

a) Infiltrates or proof of acute chest radiograph pneumonia, with proof of respiratory failure (hypoxemia, severe tachypnea)

OR

b) Confirmed laboratory influenza A infection but inadequate evidences in laboratory.

Probable definition 2:

A person dying from an unidentified acute respiratory illness who is known to be epidemiologically linked to a probable or confirmed case of H5N1 by time, place, and exposure.

Confirmed H5N1 case (notify WHO)

A person who satisfies the condition of a suspected or probable case

AND

Either of the following findings in a national, regional, or international laboratory of influenza whose H5N1 test results are recognized as confirmatory by WHO:

a) H5N1 virus isolation;
b) H5 PCR positive by tests using two separate targets of PCR, e.g. influenza A and H5 HA specific primers;
c) On test of an acute serum sample (collected 7 days or less after the start of symptom) and a convalescent serum sample, the H5N1 neutralization titer must be at least fourfold rise. The convalescent neutralizing antibody titer must also be at least 1:80;
d) In a single serum sample collected at day 14 or later after the start of symptom a microneutralization H5N1 antibody titer must be at least 1:80 and a positive in a different serological assay, for e.g. titer of at least 1:160 in a horse red blood cell HI or western blot specific to H5.

Management/Treatment

Hospitalization

H5N1 can be a serious disease in humans that needs hospitalization, isolation, and intensive care [119].

Antiviral agents

The primary therapy is the use of antiviral medication. The WHO and CDC guidelines (2015) recommend use of a neuraminidase inhibitor [92].

Amantadine

Amantadine interferes with M2 protein and affects the release of infectious viral nucleic acid [120]. It is found to be effective for both prophylaxis and short-term treatment [121]. Since most of the H5N1 virus shows resistant patterns to amantadine or rimantadine, combination therapy with oseltamivir is recommended [92].

Rimantadine (brand name: Flumadine)

It inhibits uncoating affecting viral replication. But H5N1 develops resistance to it [92].

Oseltamivir (brand name: Tamiflu)

It affects the receptor of host cells towards viral HA [92]. But the spread of oseltamivir-resistant H5N1 from 2007 to 2009 surprised the influenza community [122]. Failures of treatment have been reported in single-drug oseltamivir regimens due to resistance. There are current researches on the relative success of high-dose and/or extended oseltamivir treatment courses [83]. If the use of high-dose tends to be more effective, it will affect the supply of antiviral drugs in the case of a large epidemic, in addition to medical considerations for patients who are moderately or seriously ill [92].

Zanamivir (brand name: Relenza)

In an animal model, it shows better results but has not been tested in humans. But in severe cases, inhaled zanamivir may not be able to reach the distal airways [83, 92].

Uricosuric Agents

Probenecid can be used as adjunctive therapy [92]. But no study has been carried out to confirm the suitable dose. Studies are still going on other drugs like arbidol and peramivir [123].

Immunomodulators

Low dose corticosteroids are used to treat acute lung injury (ALI)/ARDS caused due to H5N1 [83, 124]. But the long term and high dose use may result in serious complications increasing the probability of opportunistic infections [92]. Other immunomodulators used are non-steroid anti-inflammatory drugs (NSAIDs), antipyretics, growth hormones, etc. But the use of such intervention modalities has not been proven any significant benefits [125, 126].

Miscellaneous supportive therapy in a critical case

These include oxygen therapy, ventilatory support [127], and non-ventilatory treatments for ALI/ARDS [128].

Prevention and control

Immunization

Based on different studies, three vaccines of H5N1 were licensed as Sanofi Pateur's vaccine, (monovalent killed vaccine approved by the US Food and Drug Administration (FDA), United States), Glaxo Smith Kline's vaccine-Prepandrix (approved by the European Union), and CSL Limited's vaccine-Panvax (approved by Australia) [129-131]. Even though none of the vaccines are available for the civilians yet [132]. Since the egg inoculation method is not applicable, tissue or cell culture and recombinant viruses are used for the production of vaccine and the adjuvants like aluminum hydroxide can be used to increase immunogenicity [133]. The inactivated vaccines produced by using the H5N1 was found to be immunogenic with high doses of HA [134]. But due to rapid mutation, immunization is not quite effective and practicable [135]. For travelers, prophylaxis by antiviral is not recommended, instead avoiding contact with dead birds and poultry, use of properly cooked food, and if needed use of N95 respirator mask, goggles while traveling in outbreak areas is recommended [136].

Disease management for preventing outbreaks

The most effective way to prevent an outbreak is to avoid the source of exposure. People should avoid contact with the wild as well as suspected domestic birds and even should avoid contact with contaminated feces or surface. Active surveillance and strengthening biosecurity done in poultry workers, animals, and the environment in suspected sites contribute to a great extent. [136]. One health concept or collaboration of different sectors play a vital role in early detection and prevention. Proper supervision of animal transportation, inspection on the border while transporting, and provision of isolation stations on different sites play a vital role [137]. Skilled manpower in the field of veterinary to help with identifying the diversity and emerging disease will help in early warning [136]. Stamping-Out, proper carcass disposal, and surveillance and monitoring of animal health are needed [138]. Minimizing the occupational risk of exposure and vaccination of exposed poultry workers and animals should be prioritized [136].

Pandemic preparedness

Pandemic preparedness can be implemented effectively only if it is planned by a collaboration of global, federal, and state or local levels. It requires the involvement of public health personnel, different health care professionals, researchers, and even private sectors to prepare effective measures for pandemic situations. Thus, the instant response becomes possible that will help to tackle the pandemic scenario in a relevant way [139]. Pandemic preparedness includes mass surveillance, continuous monitoring, early diagnosis, formulation of triage policies [140], development and distribution of different medical interventions like therapeutics, personal protective equipment (PPE), the rapid response of health care system, and proper interaction [139].

Precautionary measures for general publics

As a general precaution, avoiding contact with suspected wild birds, contaminated poultry products, contaminated water, and proper hand hygiene is recommended for the public. Besides, awareness programs on the public level regarding the transmission and different preventive measures should be made available by the government [136, 138].

Hospital infection control

Specific measures taken in a hospital setting not only protect the health care workers but also guide them to control the infection that in turn prevents the spread of disease in the community. Health workers must ensure the environment cleaning, proper disinfection and must be trained about occupational hazards and various modes of virus transmission [141]. N95 masks are more effective than multiple surgical masks. Pre-exposure prophylaxis should be considered if there is evidence of transmission of the virus or likely to be at risk of exposure [33, 142]. Chemoprophylaxis by oseltamivir is recommended for a person at high risk. The appropriate dose is 75mg of oseltamivir, a single dose for 7-10 days [143, 144].

Household and close contacts

There are many routes of transmission for the H5N1 virus as such there are different protective measures to avoid the infection. Proper handwashing plays a significant role in avoiding the self-inoculation of the virus, thus preventing the disease [145]. Besides, households with illness should follow extra specific measures of post-exposure chemoprophylaxis that include oseltamivir [146, 147].

Table 2. Poultry population and products in Nepal [148].

Poultry	Population
Fowl	45,171,185
Ducks	376,916
Laying hens	7,907,468
Laying ducks	174,978
Meat	Weight in metric tonnes
Chickens	40,346
Ducks	217
Eggs	Numbers in 1000
Hens	788,310
Ducks	13,060

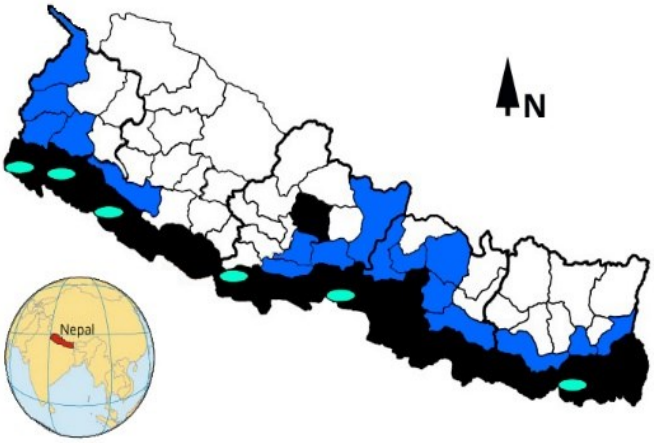
Table 3. Major events related to avian influenza in Nepal [16, 42, 150-154].

Time	Major events
2004	Surveillance of influenza started from Jhapa, eastern Nepal.
Oct 2005	First report of serologic evidence of AIV infection (H9N2) in poultry in Nepal.
2009	Domestic ducks seropositive for antibodies against H5 and H9, but not H7.
2009	With the deployment of Real-Time PCR (qPCR) at the National Public Health Laboratory (NPHL), a molecular diagnostic assay-based influenza surveillance was initiated.
Jan 2009	The first case of H5N1 detected in the Jhapa district, eastern Nepal
Jun 2009	During the 2009 pandemic influenza virus outbreak, detection and molecular characterization of pandemic influenza virus A H1N1 in a human specimen obtained at Tribhuvan International Airport.
Oct 2009	In Kathmandu valley, the community spread of pandemic H1N1 2009 was identified.
Apr 2010	Highly equipped National Influenza Centre was established at NPHL.
Apr 2011	For the isolation and characterization of influenza and parainfluenza viruses, the Madin Darby Canine Kidney (MDCK) cell line was successively cultured and propagated at NPHL.
Jun 2011	From a clinical specimen obtained and stored at NPHL, the influenza virus was isolated and characterized successively.
Nov 2011	The NPHL isolated and characterized a total of 28 influenza viruses and sent them to the WHO Collaborating Centre (WHOCC), National Institute of Infectious Diseases, Japan.
Mar 2019	The first human death by H5N1 in Nepal.

Avian influenza in the context of Nepal

Epidemiology and outbreaks history

Nepal had not observed HPAI H5N1 until 2009, although adjoining India and China reported several episodes of outbreaks. In Jhapa, a district bordering India and close to Bangladesh, the first HPAI outbreak was identified in January 2009. Although Nepal has undergone sporadic HPAI outbreaks since January 2009, until January 2012, no clinical outbreaks were identified in the capital city of Kathmandu. As per the National Agriculture census 2011, Nepal has a population of domestic poultry of around 53.6 million birds (**Table 2**). The 45% of this poultry are commercial birds and 55% are backyard birds. In Chitwan and Kathmandu Valley, the majority of the layers and broilers are raised. There were an estimated 58 hatcheries and 800 poultry producers in Nepal [148]. Most of the parent stocks are imported to Nepal from foreign countries. Occasional HPAI outbreaks has occurred in some of these stocks. In most of these countries, parent flocks are, however, subjected to strict biosecurity measures and national HPAI surveillance. It is extremely unlikely that parent stocks are contaminated with the HPAI virus. The import from Tibet is negligible. However, smuggling from India poses a greater risk of HPAI infected parent stock across the border. Also, there are minimal biosecurity practices across the industry, and migratory wild birds also use the same bodies of water used by domestically raised ducks. With arrays of wildlife reserves and national parks, migratory birds and wild birds often visit the water bodies in Nepal [149].



T**Figure 2: Map of Nepal showing risk districts and wild water bird zones.** Level of risk as represented by a different color: Black color represents the 27 high-risk districts, blue color represents the 18 medium-risk districts, white color represents the 32 low-risk districts, 6 green bubbles represent the wild water bird zones [Figure adapted from Reference 149].

Based on the national HPAI surveillance plan, Kathmandu and Chitwan are identified as high-risk disease areas in Nepal (**Figure 2**). The high-risk designation is based on the higher commercial poultry density, the higher poultry influx from other districts,

Table 4: Avian influenza outbreak history in Nepal [16].

Years	Total outbreaks	New outbreaks	District	Species	Affected population	Cases	Killed and disposed
2019	15	14-Feb	Makwanpur	Birds	Commercial layers	9,769	41,125
		28-Feb	Kathmandu	Birds	Layers	2,600	2,600
		06-Nov	Bhaktapur	Birds	Commercial broilers	1,535	3,961
		17-Mar	Kathmandu	Birds	House crow	200	0
2018	3	03-May	Chitwan	Birds	Commercial layers	1,500	12,091
		20-May	Kathmandu	Birds	Ducks	270	240
2017	4	17-Feb	Kaski	Birds	Chicken and ducks	98	297
		01-Mar	Sunsari	Birds	Commercial layers	3,650	2,550
		02-Mar	Sunsari	Birds	Swans and storks	15	0
2016	0	-	-	-	-	0	0
2015	0	-	-	-	-	0	0
2014	1	13-Feb	Sunsari	Birds	Commercial layers	570	1,430
2013	0	-	-	-	-	0	0
2012	210	27-Aug	Lalitpur	Birds	Commercial layers	2,500	0
2011	13	10-Nov	Bhaktapur	Birds	Chicken and ducks	88	308
2010	8	26-Jan	Kaski	Birds	Chicken and ducks	153	11,128
		25-Oct	Chitwan	Birds	Commercial poultry	66	11,437
2009	2	08-Jan	Jhapa	Birds	Poultry	14	24,689
Total	256					23,028	111,856

the larger free-ranging ducks, the larger natural and man-made ponds and lakes visited by migratory birds annually, the existence of live bird markets, and the lower bio-security of commercial poultry farms [16, 153]. Since the first outbreak of 2009, 256 outbreaks have been reported to OIE by Nepal. Outbreaks peaked during 2012 followed by silent 4 years. Outbreaks have been resurgent in recent years. Most of the infected population includes commercial broilers and commercial layers (**Table 4**) (**Figure 3**) [16]. Recently Epidemiology and Disease Control Division, Ministry of Health and Population confirmed the first human death by H5N1 on 2nd May 2019. The 21 years old male was admitted on 24th March 2019 and died on 29th March 2019. It has been the first and the only case of identified human death by H5N1 infection in Nepal [42, 154].

Isolation and study of influenza in human/birds/poultry

National Influenza Centre at National Public Health Laboratory (NPHL) is currently recognized by WHO and so is a member of the WHO Global Influenza Surveillance Network. Initially, influenza viruses were detected in suspected cases by Rapid Diagnostic Test (RDT), but now qPCR is employed. The center is primarily focused on collecting appropriate clinical specimens from patients, storing, transporting, and processing. Initial identification of virus type and subtype is done and isolates are forwarded to the WHO Collaborating Centre for Reference and Research on Influenza and alert the WHO Global Influenza Programme [152].

Figure 3. Map showing avian influenza outbreak clusters since

2009 in Nepal and its periphery [Figure adapted from Reference 155].

To date, 256 outbreaks have been reported by Nepal to OIE. Besides these notifications, several surveillance studies have been done in a bird population in Nepal. The first report of serologic evidence of AIV infection in poultry in Nepal was reported in October 2005 which was later determined to be the H9N2 subtype. This was clear evidence of the introduction of the virus in Nepal before 2005. The antibodies to influenza A were detected in the

chicken while ducks and pigeons were still serologically negative for the virus. This also outlines the absence of HPAI and notifiable H5 and H7 subtypes until that time. This also proved the brewing adaptation and introduction of another influenza A viruses in Nepal [150]. H5 subtype was not detected in 2007 by the RT-PCR test in suspected dead birds [156]. Similarly, domestic ducks were found seropositive for antibodies against H5 and H9 but not against H7 in 2009. Though none of the seropositive ducks were symptomatic for HPAI or LPAI virus infection [151]. H9N2 was reported in a fecal sample of migratory birds in southern Nepal. However, H7N9 and other HPAI viruses were not detected [157]. Different studies reported pandemic H1N1 in Nepal [158, 159]. Pandemic H1N1 detected in Nepal was the lineage of the novel influenza H1N1 virus (A/California/07/2009) [159].

HPAI virus has been a major threat to the Nepalese poultry industry since its outbreak in India in 2003. A substantial number of customers temporarily stopped buying chicken meat and switched to other sources of meat. The loss to entire value chain actors was estimated at NRs 1,154 million per year ($1=NRs. 73.06, the average exchange rate from 2001 to 2010) [160] from 2001 to 2010 [161]. Since the 2009 outbreak, the impacts have spilled across the country. The economic loss is even devastating. Loss of more than NRs 4.5 million has been attributed to the Pokhara bird flu outbreak alone, the third outbreak in Nepal. The commercial and backyard farmers including butchers on a small scale are primarily affected by the outbreak. Nepal government had tried to compensate for the loss during the outbreak, but the compensation offered is lower as compared to the gate price of the poultry and products [161, 162].

Outbreak Preparedness in Nepal

A pandemic imposes an increased pressure on infrastructures to contain the disease, be it a developed nation or a developing nation. Developing nations face much more stress in resource mobilization which in the case is already scarce. But in global context, pandemic help to better understand the disease and effects of preventive measures. Pandemic preparedness includes the holistic approach from virologists, epidemiologists, animal and human health professionals, military and paramilitary forces, press, media, and administrations. Continuous coordination among all parties is warranted for the execution of swift countermeasures in case of a new pandemic outbreak. But as is the case in most developing countries, this co-ordination is still lacking in Nepal [163]. Besides this, AIV is dynamically evolving and adapting to newer hosts so there is still the need to establish tests that are quick and easy to use to characterize new influenza strains [164]. Cross country movements of birds and products should strictly follow OIE recommendations. Farms should be designed to minimize the interaction with wild bird populations and there should be maximum biosecurity practices [165-167].

Public awareness level about the disease in Nepal

Different studies reported that most of the Nepalese population were aware of AIV and that poultry workers were at risk of infection. Televisions, radios, newspapers, and social media had been conveying the message to the general population about AIV. However, on preventive measures, only hand washing was widely accepted by most folks even though the majority of the population was familiar with most of the preventive behaviors. Thus, there is a strong degree of acceptance of many specific government regulation policies in public. But the implemented control measures were not considered sufficient even though preventive measures are frequently conveyed by the government (Table 4) [168, 169].

Table 4: **Excerpt of the preventive measures released by the Ministry of Health and Population, Nepal in the public interest [154].**

Frequent hand washing using soap.
Personal hygiene and cleanliness.
Environmental cleanliness.
Avoid close contact with sick poultry and wild birds.
Immediately visit nearby health institutions on suspicion of ill birds or persons in the vicinity.
Keep children far away from dead and sick birds.
Safe handling and personal safety should be considered while preparing poultry meat.
The handler should clean hands and tools after preparing meat.
Consume only well-prepared poultry meat (cooked to at least 70°C).
Don't do drugs without consultation with physicians.
Avoid close contact with dead birds or their droppings. Bury the dead birds carefully if found.

Policy in controlling outbreaks in Nepal

Between 2007 and 2011, the World Bank-funded the AIV surveillance and awareness campaign as the Avian Influenza Control Project (AICP) in Nepal. Since 2011, Nepal has continued the AICP with its internal resources. The management program focuses largely on outbreak-associated culling of poultry in tandem with washing and disinfection. control policy is mainly focused on the

outbreak-related culling of poultry in conjunction with cleaning and disinfection. However, despite of current AICP, the continuing incidence of outbreaks has raised concerns about the effectiveness of the money expended. Poultry and product export from Nepal is negligible. But domestic demand is nearly self-sufficient, though parent stocks and vaccines are imported. Thus, it is crucial to preserve the poultry industries as a pandemic can wipe out the industry owing to the small geography of the country. Thus, AICP alone cannot be fully relied upon, instead, the vaccination control program can be implemented along with bio-security, as proven effective in Pakistan [170]. Nevertheless, there is always a possibility of the circulation of the asymptomatic virus and the future spread of infection [171].

Control and management strategies in Nepal

The control and management of AIV are primarily focused on outbreak zone/s in Nepal. Once the outbreak is identified, the area is sealed and poultry production is banned for 45 days. Surveillance is intensified in the proximity of epidemic regions and other regions at higher risks. The suspected poultry are quarantined and culled. Since the first outbreak, 111,856 poultry have been killed to control the further spread. Thus, occasional outbreaks in different areas have seriously hampered the poultry industry (**Table 2**). The control and management strategies are thus targeted to outbreak areas only after the outbreak has been identified, rather than focusing on surveillance and adopting preventive measures in the outbreak prone and high-risk areas. The control measures were adopted by Nepal to curb AIV outbreaks, as reported to OIE (**Table 5**).

Table 5. Measures taken by Nepal to curb AIV outbreaks (as reported to OIE).

Control of movement in the country
Surveillance outside containment and/or protection zone
Surveillance within containment and/or protection zone
Screening
Traceability
Quarantine
Official destruction of animal products
Official disposal of carcasses, by-products, and wastes
Stamping out
Disinfection
Vaccination prohibited
No treatment of affected animals

Conclusion

More than 20 years ago, H5N1 in humans was first identified. The reservoirs and key sources of human H5N1 infections are infected birds. Human-to-human transmission is very low and initial manifestations of illness are non-specific so detailed histories along with possible travel in endemic areas should be considered for case detection. Outbreaks have been recurrent in recent years in Nepal. Commercial rapid antigen tests are insensitive and only suitable for screening. Confirmatory diagnosis can only be done by molecular techniques. Oseltamivir has been warranted for the treatment of severe cases. The understanding of epidemiology, natural history, and human H5N1 disease control is still inadequate; thus, warranting the need for co-ordination in clinical and epidemiological research among institutions globally.

Author's Contribution

BB and HP compiled the literature review. DS and SS prepared the initial draft. DS finalized the draft. All authors read and approved the final draft.

Competing Interest

The authors declare no conflict of interest or competing interest.

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References

1. Dictionary M-W: **Influenza**. In: *Merriam-Webster Dictionary*. Merriam-Webster Corporation; 2019.
2. Smith W, Andrewes CH, Laidlaw PP: **A virus obtained from influenza patients**. *The Lancet* 1933, **222**(5732):66-68. https://doi.org/10.1016/S0140-6736(00)78541-2
3. Verhoeyen M, Fang R, Jou WM, Devos R, Huylebroeck D, Saman E, Fiers W: **Antigenic drift between the haemagglutinin of the Hong Kong influenza strains A/Aichi/2/68 and A/Victoria/3/75**. *Nature* 1980, **286**(5775):771-776. https://doi.org/10.1038/286771a0
4. Abolnik C: **A current review of avian influenza in pigeons and doves (Columbidae)**. *Veterinary Microbiology* 2014, **170**(3):181-196. https://doi.org/10.1016/j.vetmic.2014.02.042
5. Tong S, Li Y, Rivailler P, Conrardy C, Castillo DAA, Chen L-M, Recuenco S, Ellison JA, Davis CT, York IA *et al*: **A distinct lineage of influenza A virus from bats**. *Proceedings of the National Academy of Sciences* 2012, **109**(11):4269. https://doi.org/10.1073/pnas.1116200109
6. Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J *et al*: **New world bats harbor diverse influenza A viruses**. *PLoS Pathog* 2013, **9**(10):e1003657-e1003657. https://doi.org/10.1371/journal.ppat.1003657
7. World organisation for Animal Health (OIE): **Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees).** In.; 2015.
8. **What is Avian Influenza.** [https://www.oie.int/en/animal-health-in-the-world/avian-influenza-portal/what-is-avian-influenza/]
9. Centers for Disease Control and Prevention (CDC): **Spread of Avian Influenza Viruses among Birds, USA**. In.; 2008.
10. Roberton SI, Bell DJ, Smith GJD, Nicholls JM, Chan KH, Nguyen DT, Tran PQ, Streicher U, Poon LLM, Chen H *et al*: **Avian influenza H5N1 in viverrids: implications for wildlife health and conservation**. *Proceedings of the Royal Society B: Biological Sciences* 2006, **273**(1595):1729-1732. https://doi.org/10.1098/rspb.2006.3549
11. WHO Global Influenza Program Surveillance Network, World Health Organization (WHO): **Evolution of H5N1 avian influenza**

viruses in Asia. *Emerg Infect Dis* 2005, **11**(10):1515. https://doi.org/10.3201/eid1110.050644
12. Melidou A, Gioula G, Exindari M, Chatzidimitriou D, Diza-Mataftsi E: **Influenza A(H5N1): an overview of the current situation**. 2009, **14**(20):19216. https://doi.org/10.2807/ese.14.20.19216-en
13. Giasuddin M, Alam J, Samad M, Al-Mamun M, Taimur J: **Highly Pathogenic Avian Influenza: Bangladesh Situation**. *Bangladesh Livestock Research Institute* 2006.
14. World Health Organization (WHO): **Geographical spread of H5N1 avian influenza in birds-update 28.** . In.; 2005.
15. UN Office for the Coordination of Humanitarian Affairs (IRIN): **Nepal: bird flu returns.** In.; 2011.
16. **World Animal Health Information Database (WAHIS Interface)-Version 1** [https://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countryreports]
17. Morin CW, Stoner-Duncan B, Winker K, Scotch M, Hess JJ, Meschke JS, Ebi KL, Rabinowitz PM: **Avian influenza virus ecology and evolution through a climatic lens**. *Environment international* 2018, **119**:241-249. https://doi.org/10.1016/j.envint.2018.06.018
18. Roche B, Lebarbenchon C, Gauthier-Clerc M, Chang CM, Thomas F, Renaud F, Van Der Werf S, Guegan JF: **Water-borne transmission drives avian influenza dynamics in wild birds: the case of the 2005–2006 epidemics in the Camargue area**. *Infection, Genetics and Evolution* 2009, **9**(5):800-805. https://doi.org/10.1016/j.meegid.2009.04.009
19. Gilbert M, Xiao X, Robinson TP: **Intensifying poultry production systems and the emergence of avian influenza in China: a 'One Health/Ecohealth'epitome**. *Archives of public health* 2017, **75**(1):48. https://doi.org/10.1186/s13690-017-0218-4
20. Liu Q, Cao L, Zhu X: **Major emerging and re-emerging zoonoses in China: a matter of global health and socioeconomic development for 1.3 billion.** *International Journal of Infectious Diseases* 2014, **25**:65-72. https://doi.org/10.1016/j.ijid.2014.04.003
21. Bui C, Bethmont A, Chughtai AA, Gardner L, Sarkar S, Hassan S, Seale H, MacIntyre CR: **A systematic review of the comparative epidemiology of avian and human influenza A H5N1 and H7N9–lessons and unanswered questions**. *Transboundary and emerging diseases* 2016, **63**(6):602-620. https://doi.org/10.1111/tbed.12327
22. de Wit E, Munster VJ, van Riel D, Beyer WEP, Rimmelzwaan GF, Kuiken T, Osterhaus ADME, Fouchier RAM: **Molecular determinants of adaptation of highly pathogenic avian influenza H7N7 viruses to efficient replication in the human host**. *Journal of Virology* 2010, **84**(3):1597-1606. https://doi.org/10.1128/JVI.01783-09
23. Chen H, Smith GJD, Zhang SY, Qin K, Wang J, Li KS, Webster RG, Peiris JSM, Guan Y: **H5N1 virus outbreak in migratory waterfowl**. *Nature* 2005, **436**(7048):191-192. https://doi.org/10.1038/nature03974
24. Gilbert M, Jambal L, Karesh WB, Fine A, Shiilegdamba E, Dulam P, Sodnomdarjaa R, Ganzorig K, Batchuluun D, Tseveenmyadag N: **Highly pathogenic avian influenza virus among wild birds in Mongolia**. *PloS one* 2012, **7**(9):e44097. https://doi.org/10.1371/journal.pone.0044097
25. Shin JH, Woo C, Wang SJ, Jeong J, An IJ, Hwang J-K, Jo S-D, Do Yu S, Choi K, Chung HM: **Prevalence of avian influenza virus in wild birds before and after the HPAI H5N8 outbreak in 2014 in South Korea**. *Journal of Microbiology* 2015, **53**(7):475-480. https://doi.org/10.1007/s12275-015-5224-z
26. Chatziprodromidou IP, Arvanitidou M, Guitian J, Apostolou T, Vantarakis G, Vantarakis A: **Global avian influenza outbreaks 2010–2016: a systematic review of their distribution, avian species and virus subtype**. *Systematic reviews* 2018, **7**(1):17. https://doi.org/10.1186/s13643-018-0691-z
27. Arnal A, Vittecoq M, Pearce-Duvet J, Gauthier-Clerc M, Boulinier T, Jourdain E: **Laridae: A neglected reservoir that could play a major role in avian influenza virus epidemiological dynamics**. *Critical reviews in microbiology* 2015, **41**(4):508-519. https://doi.org/10.3109/1040841X.2013.870967
28. Peiris JSM, de Jong MD, Guan Y: **Avian influenza virus (H5N1): a threat to human health**. *Clin Microbiol Rev* 2007, **20**(2):243-267. https://doi.org/10.1128/CMR.00037-06
29. Wrigley N: **Electron microscopy of influenza virus**. *British medical bulletin* 1979, **35**(1):35-38. https://doi.org/10.1093/oxfordjournals.bmb.a071539
30. **Influenza (Flu): Types of Influenza Viruses** [https://www.cdc.gov/flu/about/viruses/types.htm]
31. Guan Y, Peiris JSM, Lipatov AS, Ellis TM, Dyrting KC, Krauss S, Zhang LJ, Webster RG, Shortridge KF: **Emergence of multiple genotypes of H5N1 avian influenza viruses in Hong Kong SAR**. *Proceedings of the National Academy of Sciences* 2002, **99**(13):8950. https://doi.org/10.1073/pnas.132268999
32. Yamada S, Suzuki Y, Suzuki T, Le MQ, Nidom CA, Sakai-Tagawa Y, Muramoto Y, Ito M, Kiso M, Horimoto T *et al*: **Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human-type receptors**. *Nature* 2006, **444**(7117):378-382. https://doi.org/10.1038/nature05264
33. The Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5: **Avian Influenza A (H5N1) Infection in Humans**. *N Engl J Med* 2005, **353**(13):1374-1385. https://doi.org/10.1056/NEJMra052211
34. Boni MF: **Vaccination and antigenic drift in influenza**. *Vaccines (Basel)* 2008, **26**(Suppl 3):C8-C14. https://doi.org/10.1016/j.vaccine.2008.04.011
35. Treanor J: **Influenza Vaccine — Outmaneuvering Antigenic Shift and Drift**. *N Engl J Med* 2004, **350**(3):218-220. https://doi.org/10.1056/NEJMp038238
36. Webster RG, Laver WG, Air GM, Schild GC: **Molecular mechanisms of variation in influenza viruses**. *Nature* 1982, **296**(5853):115-121. https://doi.org/10.1038/296115a0
37. Hajjara SA, McIntoshb K: **The first influenza pandemic of the 21st century**. *Ann Saudi Med* 2010, **30**(1):1-10. https://doi.org/10.5144/0256-4947.59365
38. Taubenberger JK: **The Origin and Virulence of the 1918 "Spanish" Influenza Virus**. *Proc Am Philos Soc* 2006, **150**(1):86-112.
39. Waliya P, Gupta DK, Sheikh AA, Rashid R, Dar RB, Mishra A: **Avian Influenza-A pandemic threat**. *The Pharma Innovation Journal 2017*, **6**(11):526-531.
40. **Avian Influenza "at a glance"** [https://www.oie.int/en/animal-health-in-the-world/web-portal-on-avian-influenza/]
41. Duan L, Bahl J, Smith GJD, Wang J, Vijaykrishna D, Zhang LJ, Zhang JX, Li KS, Fan XH, Cheung CL: **The development and genetic diversity of H5N1 influenza virus in China, 1996–2006**. *Virology* 2008, **380**(2):243-254. https://doi.org/10.1016/j.virol.2008.07.038
42. World Health Organization (WHO): **Information on Avian Influenza A (H5N1) Identified in Human in Nepal.** In.; 2019.
43. Agriculture and Consumer Protection Department, Food and Agriculture Organisation of the United Nations: **H7N9 situation update**. In.; 2019.
44. Kang HM, Lee EK, Song BM, Jeong J, Choi JG, Jeong J, Moon OK, Yoon H, Cho Y, Kang YM: **Novel reassortant influenza A (H5N8) viruses among inoculated domestic and wild ducks, South Korea, 2014**. *Emerg Infect Dis* 2015, **21**(2):298. https://doi.org/10.3201/eid2102.141268
45. Ma MJ, Yang Y, Fang LQ: **Highly pathogenic avian H7N9 influenza viruses: recent challenges**. *Trends in microbiology* 2019, **27**(2):93-95. https://doi.org/10.1016/j.tim.2018.11.008
46. World Health Organization (WHO): **Influenza at the Human-Animal Interface, 8 December 2018–25 January 2018.** In.
47. Lazarus R, Lim PL: **Avian influenza: recent epidemiology, travel-related risk, and management**. *Current infectious disease reports* 2015, **17**(1):456. https://doi.org/10.1007/s11908-014-0456-3
48. Ozawa M, Kawaoka Y: **Taming influenza viruses**. *Virus Research* 2011, **162**(1):8-11. https://doi.org/10.1016/j.virusres.2011.09.035
49. Matrosovich M, Stech J, Klenk HD: **Influenza receptors, polymerase and host range**. *Revue Scientifique et Technique (International Office of Epizootics)* 2009, **28**(1):203-217. https://doi.org/10.20506/rst.28.1.1870
50. Basler CF, Aguilar PV: **Progress in identifying virulence determinants of the 1918 H1N1 and the Southeast Asian H5N1**

influenza A viruses. *Antiviral Res* 2008, **79**(3):166-178. https://doi.org/10.1016/j.antiviral.2008.04.006
51. Hale BG, Randall RE, Ortín J, Jackson D: **The multifunctional NS1 protein of influenza A viruses**. 2008, **89**(10):2359-2376. https://doi.org/10.1099/vir.0.2008/004606-0
52. Zhang H, Hale BG, Xu K, Sun B: **Viral and Host Factors Required for Avian H5N1 Influenza A Virus Replication in Mammalian Cells**. 2013, **5**(6):1431-1446. https://doi.org/10.3390/v5061431
53. Shinya K, Hamm S, Hatta M, Ito H, Ito T, Kawaoka Y: **PB2 amino acid at position 627 affects replicative efficiency, but not cell tropism, of Hong Kong H5N1 influenza A viruses in mice**. *Virology* 2004, **320**(2):258-266. https://doi.org/10.1016/j.virol.2003.11.030
54. Heui SS, Hoffmann E, Webster RG: **Lethal H5N1 influenza viruses escape host anti-viral cytokine responses**. *Nature Medicine* 2002, **8**(9):950-954. https://doi.org/10.1038/nm757
55. Cheung CY, Poon LLM, Lau AS, Luk W, Lau YL, Shortridge KF, Gordon S, Guan Y, Peiris JSM: **Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease**. *The Lancet* 2002, **360**(9348):1831-1837. https://doi.org/10.1016/S0140-6736(02)11772-7
56. Horimoto T, Fukuda N, Iwatsuki-Horimoto K, Guan Y, Lim W, Peiris M, Sugii S, Odagiri T, Tashiro M, Kawaoka Y: **Antigenic Differences between H5N1 Human Influenza Viruses Isolated in 1997 and 2003**. *Journal of Veterinary Medical Science* 2004, **66**(3):303-305. https://doi.org/10.1292/jvms.66.303
57. Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, Dyrting K, Rehg JE, Poon L, Guan Y, Peiris M, Webster RG: **Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks.** *Journal of Virology* 2004, **78**:4892-4901. https://doi.org/10.1128/JVI.78.9.4892-4901.2004
58. Govorkova EA, Rehg JE, Krauss S, Yen H-L, Guan Y, Peiris M, Nguyen TD, Hanh TH, Puthavathana P, Long HT *et al*: **Lethality to Ferrets of H5N1 Influenza Viruses Isolated from Humans and Poultry in 2004**. *Journal of Virology* 2005, **79**(4):2191. https://doi.org/10.1128/JVI.79.4.2191-2198.2005
59. Chantong W, Kaneene JB: **Poultry raising systems and highly pathogenic avian influenza outbreaks in Thailand: the situation, associations, and impacts**. *The Southeast Asian Journal of Tropical Medicine and Public Health* 2011, **42**:596-608.
60. Henning J, Henning KA, Morton JM, Long NT, Ha NT, Vu LT, Vu PP, Hoa DM, Meers J: **Highly pathogenic avian influenza (H5N1) in ducks and in-contact chickens in backyard and smallholder commercial duck farms in Vietnam.** *Preventive Veterinary Medicine* 2011, **101**:229-240. https://doi.org/10.1016/j.prevetmed.2010.05.016
61. Brown JD, Luttrell MP, Uhart MM, del Valle Ferreyra H, Romano MM, Rago MV, Stallknecht DE: **Antibodies to type A influenza virus in wild waterbirds from Argentina**. *Journal of Wildlife Diseases* 2010, **46**:1040-1045. https://doi.org/10.7589/0090-3558-46.3.1040
62. Chua TH: **Studies on the diagnosis, epidemiology and control of highly pathogenic H5N1 avian influenza**. Murdoch University, Perth, Australia.; 2009.
63. Pfeiffer DU, Minh PQ, Martin V, Epprecht M, Otte MJ: **An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occur-rence in Vietnam using national surveillance data.** *The Veterinary Journal* 2007, **174**:302-309. https://doi.org/10.1016/j.tvjl.2007.05.010
64. Lycett SJ, Duchatel F, Digard P: **A brief history of bird flu**. *Philos Trans R Soc Lond B Biol Sci* 2019, **374**(1775):20180257. https://doi.org/10.1098/rstb.2018.0257
65. Harfoot R, Webby RJ: **H5 influenza, a global update**. *Journal of Microbiology* 2017, **55**(3):196-203. https://doi.org/10.1007/s12275-017-7062-7
66. Smith GJD, Donis RO, World Health Organization/World Organisation for Animal Health/Food Agriculture Organization H5 Evolution Working Group: **Nomenclature updates resulting from the evolution of avian influenza A(H5) virus clades 2.1.3.2a, 2.2.1, and 2.3.4 during 2013–2014**. 2015, **9**(5):271-276. https://doi.org/10.1111/irv.12324
67. Kim S-H, Hur M, Suh J-H, Woo C, Wang S-J, Park E-R, Hwang J, An I-J, Jo S-D, Shin J-H *et al*: **Molecular characterization of highly pathogenic avian influenza H5N8 viruses isolated from Baikal teals found dead during a 2014 outbreak in Korea**. *J Vet Sci* 2016, **17**(3):299-306. https://doi.org/10.4142/jvs.2016.17.3.299
68. Miller RS, Sweeney SJ, Akkina JE, Saito EK: **Potential Intercontinental Movement of Influenza A(H7N9) Virus into North America by Wild Birds: Application of a Rapid Assessment Framework**. *Transboundary and emerging diseases* 2015, **62**(6):650-668. https://doi.org/10.1111/tbed.12213
69. Couacy-Hymann E, Kouakou VA, Aplogan GL, Awoume F, Kouakou CK, Kakpo L, Sharp BR, McClenaghan L, McKenzie P, Webster RG *et al*: **Surveillance for influenza viruses in poultry and swine, west Africa, 2006-2008**. *Emerging infectious diseases* 2012, **18**(9):1446-1452. https://doi.org/10.3201/eid1809.111296
70. WHO/OIE/FAO H5N1 Evolution Working Group: **Continued evolution of highly pathogenic avian influenza A (H5N1): updated nomenclature**. *Influenza Other Respir Viruses* 2012, **6**(1):1-5. https://doi.org/10.1111/j.1750-2659.2011.00298.x
71. Peiris JSM, Yu WC, Leung CW, Cheung CY, Ng WF, Nicholls JM, Ng TK, Chan KH, Lai ST, Lim WL *et al*: **Re-emergence of fatal human influenza A subtype H5N1 disease.** *The Lancet* 2004, **363**(9409):617-619. https://doi.org/10.1016/S0140-6736(04)15595-5
72. de Jong MD, Cam BV, Qui PT, Hien VM, Thanh TT, Hue NB, Beld M, Phuong LT, Khanh TH, Chau NVV *et al*: **Fatal Avian Influenza A (H5N1) in a Child Presenting with Diarrhea Followed by Coma**. *N Engl J Med* 2005, **352**(7):686-691. https://doi.org/10.1056/NEJMoa044307
73. Uiprasertkul M, Puthavathana P, Sangsiriwut K, et al: **Influenza A H5N1 replication sites in humans.** *Emerg Infect Dis* 2005, **11**:1036-1041. https://doi.org/10.3201/eid1107.041313
74. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y: **Influenza virus receptors in the human airway**. *Nature* 2006, **440**(7083):435-436. https://doi.org/10.1038/440435a
75. Pillai SPS, Lee CW: **Species and age related differences in the type and distribution of influenza virus receptors in different tissues of chickens, ducks and turkeys**. *Virology Journal* 2010, **7**(1):5. https://doi.org/10.1186/1743-422X-7-5
76. Skehel JJ, Wiley DC: **Receptor Binding and Membrane Fusion in Virus Entry: The Influenza Hemagglutinin**. *Annual Review of Biochemistry* 2000, **69**(1):531-569. https://doi.org/10.1146/annurev.biochem.69.1.531
77. Neumann G, Kawaoka Y: **Host range restriction and pathogenicity in the context of influenza pandemic**. *Emerg Infect Dis* 2006, **12**(6):881-886. https://doi.org/10.3201/eid1206.051336
78. To KF, Chan PK, Chan KF, Lee WK, Lam WY, Wong KF, Tang NL, Tsang DN, Sung RY, Buckley TA *et al*: **Pathology of fatal human infection associated with avian influenza A H5N1 virus**. *Journal of medical virology* 2001, **63**(3):242-246. https://doi.org/10.1002/1096-9071(200103)63:3<242::AID-JMV1007>3.0.CO;2-N
79. Weinstein RA, Bridges CB, Kuehnert MJ, Hall CB: **Transmission of influenza: implications for control in health care settings**. *Clinical infectious diseases* 2003, **37**(8):1094-1101. https://doi.org/10.1086/378292
80. Mounts AW, Kwong H, Izurieta HS, Ho Y-y, Au T-k, Lee M, Bridges CB, Williams SW, Mak KH, Katz JM *et al*: **Case-Control Study of Risk Factors for Avian Influenza A (H5N1) Disease, Hong Kong, 1997**. *The Journal of Infectious Diseases* 1999, **180**(2):505-508. https://doi.org/10.1086/314903
81. Thanawongnuwech R, Amonsin A, Tantilertcharoen R, Damrongwatanapokin S, Theamboonlers A, Payungporn S, Nanthapornphiphat K, Ratanamungklanon S, Tunak E, Songserm T *et al*: **Probable tiger-to-tiger transmission of avian influenza H5N1**. *Emerg Infect Dis* 2005, **11**(5):699-701. https://doi.org/10.3201/eid1105.050007
82. Hayden F, Croisier A: **Transmission of Avian Influenza Viruses to and between Humans**. *The Journal of Infectious Diseases* 2005, **192**(8):1311-1314. https://doi.org/10.1086/444399
83. Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian

Influenza A (H5N1) Virus, Adbdel-Ghafar AN, Chotpitayasunondh T, Gao Z, Hayden FG, Nguyen DH, et al: **Update on Avian Influenza A (H5N1) Virus Infection in Humans**. *N Engl J Med* 2008, **358**(3):261-273. https://doi.org/10.1056/NEJMra0707279

84. Chotpitayasunondh T, Ungchusak K, Hanshaoworakul W, Chunsuthiwat S, Sawanpanyalert P, Kijphati R, Lochindarat S, Srisan P, Suwan P, Osotthanakorn Y *et al*: **Human disease from influenza A (H5N1), Thailand, 2004**. *Emerg Infect Dis* 2005, **11**(2):201-209. https://doi.org/10.3201/eid1102.041061
85. Kandun IN, Wibisono H, Sedyaningsih ER, Yusharmen, Hadisoedarsuno W, Purba W, Santoso H, Septiawati C, Tresnaningsih E, Heriyanto B *et al*: **Three Indonesian Clusters of H5N1 Virus Infection in 2005**. *N Engl J Med* 2006, **355**(21):2186-2194. https://doi.org/10.1056/NEJMoa060930
86. Oner AF, Bay A, Arslan S, Akdeniz H, Sahin HA, Cesur Y, Epcacan S, Yilmaz N, Deger I, Kizilyildiz B *et al*: **Avian Influenza A (H5N1) Infection in Eastern Turkey in 2006**. *N Engl J Med* 2006, **355**(21):2179-2185. https://doi.org/10.1056/NEJMoa060601
87. Hien TT, Liem NT, Dung NT, San LT, Mai PP, Chau NvV, Suu PT, Dong VC, Mai LTQ, Thi NT *et al*: **Avian influenza A (H5N1) in 10 patients in Vietnam**. *N Engl J Med* 2004, **350**(12):1179-1188. https://doi.org/10.1056/NEJMoa040419
88. Yuen KY, Chan PKS, Peiris M, Tsang DNC, Que TL, Shortridge KF, Cheung PT, To WK, Ho ETF, Sung R *et al*: **Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus**. *The Lancet* 1998, **351**(9101):467-471. https://doi.org/10.1016/S0140-6736(98)01182-9
89. Yusuke SK, Kiyoshi F, Kazuo S: **Mechanism of a Mutation in Non-Structural Protein 1 Inducing High Pathogenicity of Avian Influenza Virus H5N1**. *Protein & Peptide Letters* 2016, **23**(4):372-378. https://doi.org/10.2174/0929866523666160204124406
90. Apisarnthanarak A, Kitphati R, Thongphubeth K, Patoomanunt P, Anthanont P, Auwanit W, Thawatsupha P, Chittaganpitch M, Saeng-Aroon S, Waicharoen S *et al*: **Atypical avian influenza (H5N1)**. *Emerg Infect Dis* 2004, **10**(7):1321-1324.
91. Sandrock C, Kelly T: **Clinical review: update of avian influenza A infections in humans**. *Critical care* 2007, **11**(2):209-209. https://doi.org/10.1186/cc5675
92. World Health Organization (WHO): **Clinical management of human infection with avian influenza A (H5N1) virus.** In.; 2015.
93. World Health Organization (WHO): **Update: WHO-confirmed human cases of avian influenza A (H5N1) infection, 25 November 2003-24 November 2006**. *Weekly Epidemiological Record* 2007, **82**(06):41-47.
94. **Avian Influenza (Bird Flu)** [http://emedicine.medscape.com/article/250002]
95. World Health Organization (WHO): **Epidemiology of WHO-confirmed human cases of avian influenza A (H5N1) infection**. *Weekly Epidemiological Record* 2006, **81**(26):249-257.
96. Gruber PC, Gomersall CD, Joynt GM: **Avian influenza (H5N1): implications for intensive care**. *Intensive Care Med* 2006, **32**(6):823-829. https://doi.org/10.1007/s00134-006-0148-z
97. Chan PKS: **Outbreak of Avian Influenza A(H5N1) Virus Infection in Hong Kong in 1997**. *Clinical Infectious Diseases* 2002, **34**(Supplement_2):S58-S64. https://doi.org/10.1086/338820
98. Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amonsin A, Payungporn S, Noppornpanth S, Wattanodorn S, Theamboonlers A, Tantilertcharoen R: **Avian influenza H5N1 in tigers and leopards**. *Emerg Infect Dis* 2004, **10**(12):2189. https://doi.org/10.3201/eid1012.040759
99. Regional Office for South-East Asia, World Health Organization (WHO): **Guidelines on laboratory diagnosis of avian influenza**. In. New Delhi: WHO Regional Office for South-East Asia; 2007.
100. World Health Organization (WHO): **Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection : guide for field operations**. In. Geneva: World Health Organization; 2006.
101. Madeley CR, World Health Organization (WHO): **Guide to the collection and transport of virological specimens : including chlamydial and rickettsial specimens**. In. Geneva: World Health Organization; 1977.
102. **Specimen Collection and Testing for patients with novel influenza A viruses with potential to cause severe disease in humans** [https://www.cdc.gov/flu/avianflu/severe-potential.htm]
103. Ng EKO, Cheng PKC, Ng AYY, Hoang TL, Lim WWL: **Influenza A H5N1 detection**. *Emerg Infect Dis* 2005, **11**(8):1303-1305. https://doi.org/10.3201/eid1108.041317
104. Poon LLM, Leung CSW, Chan KH, Lee JHC, Yuen KY, Guan Y, Peiris JSM: **Detection of human influenza A viruses by loop-mediated isothermal amplification**. *J Clin Microbiol* 2005, **43**(1):427-430. https://doi.org/10.1128/JCM.43.1.427-430.2005
105. Imai M, Ninomiya A, Minekawa H, Notomi T, Ishizaki T, Tashiro M, Odagiri T: **Development of H5-RT-LAMP (loop-mediated isothermal amplification) system for rapid diagnosis of H5 avian influenza virus infection**. *Vaccines (Basel)* 2006, **24**(44-46):6679-6682. https://doi.org/10.1016/j.vaccine.2006.05.046
106. Chan KH, Lam S, Puthavathana P, Nguyen T, Long H, Pang C, Chan KM, Cheung C, Seto WH, Peiris JS: **Comparative analytical sensitivities of six rapid influenza A antigen detection test kits for detection of influenza A subtypes H1N1, H3N2 and H5N1**. *Journal of clinical virology* 2007, **38**:169-171. https://doi.org/10.1016/j.jcv.2006.11.010
107. Kida H, Ito T, Yasuda J, Shimizu Y, Itakura C, Shortridge KF, Kawaoka Y, Webster RG: **Potential for transmission of avian influenza viruses to pigs**. *Journal of General Virology* 1994, **75**(9):2183-2188. https://doi.org/10.1099/0022-1317-75-9-2183
108. Lu BL, Webster RG, Hinshaw VS: **Failure to detect hemagglutination-inhibiting antibodies with intact avian influenza virions**. *Infect Immun* 1982, **38**(2):530-535. https://doi.org/10.1128/IAI.38.2.530-535.1982
109. Beare A, Webster R: **Replication of avian influenza viruses in humans**. *Archives of virology* 1991, **119**(1-2):37-42. https://doi.org/10.1007/BF01314321
110. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC: **Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine**. *Virus research* 2004, **103**:91-95. https://doi.org/10.1016/j.virusres.2004.02.019
111. Rowe T, Abernathy RA, Hu-Primmer J, Thompson WW, Lu X, Lim W, Fukuda K, Cox NJ, Katz JM: **Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays**. *J Clin Microbiol* 1999, **37**(4):937-943. https://doi.org/10.1128/JCM.37.4.937-943.1999
112. Bridges CB, Katz JM, Seto WH, Chan PKS, Tsang D, Ho W, Mak KH, Lim W, Tam JS, Clarke M *et al*: **Risk of Influenza A (H5N1) Infection among Health Care Workers Exposed to Patients with Influenza A (H5N1), Hong Kong**. *The Journal of Infectious Diseases* 2000, **181**(1):344-348. https://doi.org/10.1086/315213
113. Bridges CB, Lim W, Hu-Primmer J, Sims L, Fukuda K, Mak KH, Rowe T, Thompson WW, Conn L, Lu X *et al*: **Risk of Influenza A (H5N1) Infection among Poultry Workers, Hong Kong, 1997–1998**. *The Journal of Infectious Diseases* 2002, **185**(8):1005-1010. https://doi.org/10.1086/340044
114. Katz JM, Lim W, Bridges CB, Rowe T, Hu-Primmer J, Lu X, Abernathy RA, Clarke M, Conn L, Kwong H: **Antibody response in individuals infected with avian influenza A (H5N1) viruses and detection of anti-H5 antibody among household and social contacts**. *The Journal of Infectious Diseases* 1999, **180**(6):1763-1770. https://doi.org/10.1086/315137
115. **Avian influenza A (H5N1) virus infection** [https://online.epocrates.com/diseases/45535/Avian-influenza-A-H5N1-virus-infection/Differential-Diagnosis]
116. Lai S, Qin Y, Cowling BJ, Ren X, Wardrop NA, Gilbert M, Tsang TK, Wu P, Feng L, Jiang H *et al*: **Global epidemiology of avian influenza A H5N1 virus infection in humans, 1997–2015: a systematic review of individual case data**. *The Lancet Infectious Diseases* 2016, **16**(7):e108-e118. https://doi.org/10.1016/S1473-3099(16)00153-5
117. **Asian H5N1 Case Defination** [https://www.cdc.gov/flu/avianflu/h5n1/case-definitions.htm]
118. **WHO case definitions for human infections with influenza A(H5N1) virus**

[https://www.who.int/influenza/resources/documents/case_definition2006_08_29/en/]
119. White NJ, Webster RG, Govorkova EA, Uyeki TM: **What is the optimal therapy for patients with H5N1 influenza?** *PLoS Med* 2009, **6**(6):e1000091-e1000091. https://doi.org/10.1371/journal.pmed.1000091
120. Jefferson T, Jones MA, Doshi P, Del Mar CB, Hama R, Thompson MJ, Spencer EA, Onakpoya IJ, Mahtani KR, Nunan D *et al*: **Neuraminidase inhibitors for preventing and treating influenza in adults and children**. *Cochrane Database of Systematic Reviews* 2014, **2014**(4). https://doi.org/10.1002/14651858.CD008965.pub4
121. Allen UD, Aoki FY, Stiver HG: **The use of antiviral drugs for influenza: recommended guidelines for practitioners**. *Canadian Journal of Infectious Diseases & Medical Microbiology* 2006, **17**(5):273-284. https://doi.org/10.1155/2006/165940
122. Poland GA, Jacobson RM, Ovsyannikova IG: **Influenza virus resistance to antiviral agents: a plea for rational use**. *Clinical infectious diseases* 2009, **48**(9):1254-1256. https://doi.org/10.1086/598989
123. Hill G, Cihlar T, Oo C, Ho ES, Prior K, Wiltshire H, Barrett J, Liu B, Ward P: **The Anti-Influenza Drug Oseltamivir Exhibits Low Potential to Induce Pharmacokinetic Drug Interactions via Renal Secretion—Correlation of in Vivo and in Vitro Studies**. *Drug Metabolism and Disposition* 2002, **30**(1):13. https://doi.org/10.1124/dmd.30.1.13
124. Hui DS, Lee N, Chan PK, Beigel JH: **The role of adjuvant immunomodulatory agents for treatment of severe influenza**. *Antiviral Res* 2018, **150**:202-216. https://doi.org/10.1016/j.antiviral.2018.01.002
125. World Health Organization (WHO): **WHO rapid advice guidelines on pharmacological management of humans infected with avian influenza A (H5N1) virus**. In. Geneva: World Health Organization; 2006.
126. Schünemann HJ, Hill SR, Kakad M, Bellamy R, Uyeki TM, Hayden FG, Yazdanpanah Y, Beigel J, Chotpitayasunondh T, Del Mar C *et al*: **WHO Rapid Advice Guidelines for pharmacological management of sporadic human infection with avian influenza A (H5N1) virus**. *The Lancet Infectious Diseases* 2007, **7**(1):21-31. https://doi.org/10.1016/S1473-3099(06)70684-3
127. World Health Organization (WHO): **WHO interim guidelines on clinical management of humans infected by influenza A (H5N1)**. 2004.
128. The ARDS Network: **Comparison of Two Fluid-Management Strategies in Acute Lung Injury**. *N Engl J Med* 2006, **354**(24):2564-2575. https://doi.org/10.1056/NEJMoa062200
129. **Australia approves CSL's H5N1 vaccine.** [http://www.cidrap.umn.edu/news-perspective/2008/06/australia-approves-csls-h5n1-vaccine]
130. **FDA Approves First Adjuvanted Vaccine for H5N1 Bird Flu.** [https://www.medscape.com/viewarticle/814914]
131. **EU license first pre-pandemic bird flu vaccine London** [https://www.reuters.com/article/us-birdflu-glaxo/eu-licenses-first-pre-pandemic-bird-flu-vaccine-idUSL1644576720080519]
132. Elegant S: **Is a human pandemic next?** In: *Time*. vol. 163; 2004: 14-20.
133. Baz M, Luke CJ, Cheng X, Jin H, Subbarao K: **H5N1 vaccines in humans**. *Virus research* 2013, **178**(1):78-98. https://doi.org/10.1016/j.virusres.2013.05.006
134. Altman L: **Avian flu drug works in first tests**. *New York Times* 2005.
135. **Bird Flu.** [https://www.britannica.com/science/bird-flu]
136. **Prevention and treatment of Avian Influenza A Viruses in People** [https://www.cdc.gov/flu/avianflu/prevention.htm]
137. Lam TT-Y, Zhou B, Wang J, Chai Y, Shen Y, Chen X, Ma C, Hong W, Chen Y, Zhang Y *et al*: **Dissemination, divergence and establishment of H7N9 influenza viruses in China**. *Nature* 2015, **522**(7554):102-105. https://doi.org/10.1038/nature14348
138. Lemon SM, Mahmoud A, Mack A, Knobler SL: **The threat of pandemic influenza: are we ready? workshop summary**: National Academies Press; 2005.
139. WHO Global Influenza Programme, World Health Organization (WHO): **Pandemic influenza preparedness and response: a WHO guidance document**. In. Geneva: World Health Organization; 2009.
140. Gomersall CD, Loo S, Joynt GM, Taylor BL: **Pandemic preparedness**. 2007, **13**(6):742-747. https://doi.org/10.1097/MCC.0b013e3282f1bafd
141. Hans C: **Infection control practices for avian influenza in healthcare facilities.** *J Indian Med Assoc* 2006, **104**(7):388-392.
142. Derrick JL, Gomersall CD: **Protecting healthcare staff from severe acute respiratory syndrome: filtration capacity of multiple surgical masks**. *Journal of Hospital Infection* 2005, **59**(4):365-368. https://doi.org/10.1016/j.jhin.2004.10.013
143. Hayden FG, Belshe R, Villanueva C, Lanno R, Hughes C, Small I, Dutkowski R, Ward P, Carr J: **Management of Influenza in Households: A Prospective, Randomized Comparison of Oseltamivir Treatment With or Without Postexposure Prophylaxis**. *The Journal of Infectious Diseases* 2004, **189**(3):440-449. https://doi.org/10.1086/381128
144. Welliver R, Monto AS, Carewicz O, Schatteman E, Hassman M, Hedrick J, Jackson HC, Huson L, Ward P, Oxford JS *et al*: **Effectiveness of Oseltamivir in Preventing Influenza in Household ContactsA Randomized Controlled Trial**. *JAMA* 2001, **285**(6):748-754. https://doi.org/10.1001/jama.285.6.748
145. World Health Organization (WHO): **WHO guidelines on hand hygiene in health care (advanced draft): a summary: clean hands are safer hands**. In.: Geneva: World Health Organization; 2005.
146. World Health Organization (WHO): **Human cases of avian influenza A(H5N1) in North-West Frontier Province, Pakistan, October-November 2007**. In., vol. 83; 2008: 359-364.
147. **Interim Guidance on Follow-up of Close Contacts of Persons Infected with Novel Influenza A Viruses Associated with Severe Human Disease and on the Use of Antiviral Medications for Chemoprophylaxis** [https://www.cdc.gov/flu/avianflu/novel-av-chemoprophylaxis-guidance.htm]
148. Ministry of Agricultural Development, Nepal: **Statistical Information on Nepalese Agriculture.** In.; 2011/2012.
149. Department of Livestock Services, Ministry of Agriculture and Cooperatives: **National Surveillance Plan for Highly Pathogenic Avian Influenza (HPAI).** In.; 2008.
150. Pant GR, Selleck PW: **Surveillance for Avian Influenza in Nepal 2004-2005 (Vigilancia epidemiológica para influenza aviar en Nepal durante los años 2004 y 2005)**. *Avian Diseases* 2007, **51**(1):352-354. https://doi.org/10.1637/7649-051806R.1
151. Pandey S, Sherchand SP, Manandhar P, Pokhrel N, Globig A, Sherchan S: **Serological Surveillance of Avian Influenza Virus in Nepal.** *Journal of Human Virology and Retrovirology* 2016, **4**(1):00120. https://doi.org/10.15406/jhvrv.2016.04.00120
152. **National Influenza Center** [http://nphl.gov.np/department/influenza]
153. Chaudhary S, Pahwa VK: **Avain influenza**. *Journal of Universal College of Medical Sciences* 2013, **1**(3):1-2. https://doi.org/10.3126/jucms.v1i3.8750
154. Epidemiology and Disease Control Division, Department of Health Services, Nepal: **Press release on Influenza A (H5N1) by Ministry of Health and Population.** In.; 2019.
155. **Disease outbreak maps. World Animal Health Information Database (WAHIS Interface) – Version 1.** [https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseaseoutbreakmaps?disease_type_hidden=0&disease_id_hidden=15&selected_disease_name_hidden=Highly+path.+avian+influenza+%28-+%29+&disease_type=0&disease_id_terrestrial=15&disease_id_aquatic=-999&selected_start_day=1&selected_start_month=1&selected_start_year=2013&selected_end_day=1&selected_end_month=12&selected_end_year=2013&newlang=en]
156. Dhakal D, Dulal P, Shrestha RM, Manandhar S, Lamichane J: **Study of Prevalence of Bird flu by using RT-PCR at Central Veterinary Laboratory, Nepal, 2007.** *BSN E-Bulletin* 2009, **1**.
157. Karmacharya D, Manandhar S, Sharma A, Bhatta T, Adhikari P, Sherchan AM, Shrestha B, Bista M, Rajbhandari R, Oberoi M *et al*: **Surveillance of Influenza A Virus and Its Subtypes in Migratory**

Wild Birds of Nepal. *PloS one* 2015, **10**(7):e0133035. https://doi.org/10.1371/journal.pone.0133035
158. Mahato RK, Bhandari GP, Shrestha JM, Basnet P: **Pandemic Influenza A (H1N1) 2009 outbreak investigation in Nepal**. *Journal of Nepal Health Research Council* 2011, **8**(17):75-77. https://doi.org/10.33314/jnhrc.v0i0.229
159. Adhikari BR, Shakya G, Upadhyay BP, Prakash Kc K, Shrestha SD, Dhungana GR: **Outbreak of pandemic influenza A/H1N1 2009 in Nepal**. *Virology Journal* 2011, **8**(1):133. https://doi.org/10.1186/1743-422X-8-133
160. **Foreign Exchange Rates Fixed by Nepal Rastra Bank (NRB)** [https://archive.nrb.org.np/fxmexchangerate.php]
161. Ghimire YN, Karki NPS, Shrestha K: **Economic Loss due to Outbreak of Highly Pathogenic Avian Influenza in the Nepalese Commercial Poultry**. *Nepal Agric Res J* 2012, **12**:84-90.
162. Karki S: **Effects of highly pathogenic avian influenza H5N1 outbreak In nepal from financial and social perspectives: a case study.** *Nepalese Vet J* 2017, **34**:26-35. https://doi.org/10.3126/nvj.v34i0.22861
163. Dharma K, Chakraborty S, Tiwari R, Kumar A, Rahal A, Latheef S, Wani M, Kapoor S: **Avian/Bird Flu Virus: Poultry Pathogen Having Zoonotic and Pandemic Threats: A Review.** *J Med Sci* 2013, **13**(5):301-315. https://doi.org/10.3923/jms.2013.301.315
164. Mak PW, S. Jayawardena S, Poon LL: **The evolving threat of influenza viruses of animal origin and the challenges in developing appropriate diagnostics.** *Clin Chem* 2012, **58**:1527-1533. https://doi.org/10.1373/clinchem.2012.182626
165. World Health Organization (WHO): **Avian influenza A (H5N1)-update 31: Situation (poultry) in Asia: Need for a long-term response, comparision with previous outbreaks. Epidemic and Pandemic Alert and Response (EPR).** In.; 2004.
166. Dharma K, Chauhan RS, Kataria JM, Mahendran M, Tomar S: **Avian Influenza: The current perspectives.** *J Immunol Immunopathol* 2005, **7**:1-33.
167. Dharma KM, Mahendran M, Tomar S: **Pathogens transmitted by migratory birds: Threat perceptions to poultry health and production**. *Int J Poult Sci* 2008, **7**:516-525. https://doi.org/10.3923/ijps.2008.516.525
168. Neupane D, Khanal V, Ghimire K, Aro AR, Leppin A: **Knowledge, attitudes and practices related to avian influenza among poultry workers in Nepal: a cross sectional study**. *BMC Infectious Diseases* 2012, **12**(1):76. https://doi.org/10.1186/1471-2334-12-76
169. Sah JK, Chiluwal S, Yadav SK, Jha D: **A study on knowledge and preventive practices related to Avian Influenza among Higher Secondary School Students of Rajbiraj Municipality, Nepal.** *Al Ameen J Med Sci* 2017, **10**(4):276-280. https://doi.org/10.15406/mojph.2016.04.00091
170. Naeem K: **The avian influenza H7N3 outbreak in South Central Asia.** *Avian Diseases* 2003, **47**:31-35.
171. Ellis TM, Leung CY, Chow MK, Bissett LA, Wong W, Guan Y, Malik Peiris J: **Vaccination of chickens against H5N1 avian influenza in the face of an outbreak interrupts virus transmission.** *Avian Pathology* 2004, **33**:405-412. https://doi.org/10.1080/03079450410001724012