Review Article

Current information of H9N2 virus zoonotic infection and its emerging pandemic potential: A review

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ABSTRACT

H9N2 avian influenza viruses cause worldwide infections in animals including humans and show a threat as a pandemic infection. Since 1998 more than 59 cases including one death due to H9N2 infection had been reported worldwide and the majority of confirmed cases were young children. Due to the large host variety, tolerance to both poultry and mammals and widespread gene reassortment, H9N2 viruses played a crucial role in worldwide infection. In this review, we discuss the current worldwide infection of H9N2 avian influenza viruses as well as their host range, pathogenesis, epidemiology, diagnosis, control, and its pandemic potential.

Key words: H9N2, infection, influenza, pandemic, reassortment, zoonotic

INTRODUCTION

Avian influenza virus belongs to the family orthomyxoviridae class A influenza virus.[1] The avian influenza virus genome consists of eight segments of negative-sense, single-stranded RNA encoding at least 10 proteins: two surface glycoproteins (hemagglutinin [HA] and neuraminidase [NA]), nucleoprotein, three polymerase essential proteins (PB2, PB1, and acidic polymerase), two matrix proteins (M1 and M2) and two nonstructural proteins (NS1 and NS2).[2] Avian influenza virus is categorized into subtypes based on the antigenic variations between the glycoprotein HA and NA. Influenza included 16 HA subtypes (H1 to H16) and nine NA subtypes (N1 to N9) A virus and viruses of all subtypes have been isolated from avian species.[3] Domestic poultry infection results in complex clinical syndromes of varying severity depending on the strain of the virus, the host species and the existence of secondary pathogens.[4] Highly pathogenic avian influenza viruses in chickens (H5 and H7 subtype), show high pathogenicity and contain polybasic cleavage sites which allow systematic virus replication in birds. However, low pathogenicity avian influenza viruses in chickens (H9N2 subtype), show low pathogenicity and contain mono, di or tri-basic cleavage site in HA which allows the cleavage of HA restricting the virus to respiratory and gastrointestinal tracts.[5]

Influenza A viruses give rise to different subtypes such as H1N1, H5N6, or H9N2 by different combinations of HA and NA surface proteins. Wild waterfowl and sea birds are the natural hosts of nearly all subtypes of influenza virus except H17N10 and H18N11 subtypes which are found in

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bats.[6] In 1966, first time, these viruses were isolated from Turkey (A/turkey/Wisconsin/1/1966).[7] Influenza virus transmits through aerosol, droplets, fecal-oral route and direct contact.[8] Studies show that respiratory and contact transmissions are probably the primary routes of transmission in case of H9N2 infection. In domestic poultry, the infection of H9N2 is commonly linked to reduced feed intake, less egg production, moderate respiratory symptoms, and low mortality rate.[9]

In the past 10 years, the epidemic infection of H9N2 in commercial poultry flocks results in severe clinical symptoms, high mortality rate, and huge loss in production.[10,11] Human cases of influenza H9N2 virus infection have been observed in Hong Kong, China, Bangladesh, Pakistan, Egypt, Oman, and India.[12-16] H9N2 outbreaks in commercial chickens have been reported recently in Asia (India, Pakistan, China, Hong Kong, Korea, and Japan), the Middle East (Saudi Arabia, United Arab Emirates, Jordan, Iran, Iraq, Lebanon and Kuwait), and African countries (Egypt, Tunisia, Libya, and South Africa).[17] H9N2 influenza viruses emerge as drift variants due to constant mutations which acquire more virulence gradually.[18] In China, an influenza A (H9N2) virus (B51) was isolated in 2017 from migratory waterfowl. This B51 was the novel reassortment influenza virus that contains the gene segments from North American wild bird influenza viruses and human H7N4 virus.[19] H9N2 viruses provide internal genes to the H5N1, H7N9, H10N8 and H5N6 viruses.[20-23]

A recent infection of H9N2 was detected in a 17-month-old boy from Melghat District, Maharashtra State, India. The strain of this H9N2 virus (A/India/TCM2581/2019) confirmed the mixed lineage of G1 and H7N3. This recent case of H9N2 infection in a 17-month-old boy in India can be a threat to human health in India and there is an urgent need for the surveillance in this case.[16]

PATHOGENESIS

The subtypes of avian influenza viruses are determined by the antigenic difference between HA and NA surface glycoproteins; however, the HA largely determines the virulence of the virus in poultry.[24] A mutation in viral surface protein HA in Tunisian outbreaks strain isolated in 2011 allows the virus to bind to sialic receptor $\alpha^{[2,6]}$ of humans which made the virus highly pathogenic.[25,26] The widespread subtype of influenza viruses in Chinese chickens was H9N2 which caused significant economic losses for the poultry industry, including those under long-term vaccination programs. Recent human infections with avian influenza virus have confirmed that H9N2 is the donor gene for H7N9 and H10N8 viruses which also infect humans. In China, the H9N2 virus was detected in a number of avian species including pigeon, duck, quail, pertussis, pheasant, chicken, egret, chukar, and silky chicken. Aerosol, droplet particles, oral-facial route and direct touch can transmit the H9N2 influenza virus. Transmission of contact relies on the transfer of particles directly to mucous membranes, or intermediate through fomite. After the infection, chickens showed no clinical sign generally but some of them showed ruffled feathers and depression. The virus inside the trachea replicates itself; this makes chickens more vulnerable to secondary infections, in particular, co-infections with *Escherichia coli* or other pathogens. Furthermore, when ventilation is low, trachea, and bronchi are easily embolized by mucus which leads to severe respiratory disease and death.[27,28] Live bird markets function as hubs for poultry traders and their birds are a major component of the disease transmission pathway, shown to keep avian influenza virus spread among poultry as well as facilitate zoonotic infections.[29,30]

EPIDEMIOLOGY

The H9N2 influenza outbreak began in northern Israel, from where the epizootic spread throughout the world. The diagnostics used the reverse transcription polymerase chain reaction (PCR) in addition to the standard serological studies. H9N2 virus causes low pathogenicity when it infects individually without other respiratory pathogens. However, it causes high mortality when co-infected with other pathogens. A previous study revealed that the co-infection of H9N2 (A/chicken/1618F/2016) with *E. coli* O78 in specific-pathogen-free chickens can increase the mortality rates to a percentage of 20%, which leads to economic losses up to 75% in chicken flocks.[31-34] Influenza A subtype H9N2 viruses are now known to be prevalent in poultry. Previous studies suggest that the H5N1 viruses responsible for the highly pathogenic disease that occurred in Hong Kong in 1997 were reassortant from avian H9N2 viruses. Getting diagnostic tools for identifying H9N2 viruses is important for the preparedness for pandemic influenza.

DIAGNOSIS

H9N2 has been reported from indigenous poultry. Seroepidemiological study was undertaken among poultry workers in Pune, India, to understand the prevalence of antibodies against avian influenza H9N2. Serum samples of poultry workers were tested by hemagglutination inhibition and micro neutralization assays for the presence of antibodies against avian influenza H9N2 virus. Low prevalence of antibodies against H9N2 virus was found in this study but further serological studies are urgently required for poultry workers in India.[35] Bangladesh has reported high levels of highly pathogenic avian influenza H5N1 outbreaks in poultry.[36,37] In the poultry in Bangladesh, a natural reassortant HPAI (H5N1) virus containing a H9N2-PBI gene was identified. Serological studies showed that a high rate of seropositivity was found among poultry workers in different enzootic countries including China, Egypt, Cambodia, Iran, Vietnam, Hong Kong, Pakistan, Thailand, and India.[5,38]

Recently, a method to detect H9N2 avian influenza is developed in which monoclonal antibodies was developed against H9N2 virus and applied a double-antibody sandwich immunosorbent enzyme-linked assay (DAS-ELISA) to detect the H9N2 viral antigen. The specificity and sensitivity of DAS-ELISA was found to be >98%.[39] A highly sensitive real time immuno-PCR method is used for detecting H9N2 virus. The assay applies aptamers as ligands to capture and detect the virus.[26]

PREVENTION AND CONTROL

H9N2 caused economic loss in several nations, including China, UAE, Morocco, Israel, South Korea, Iran, Egypt, and Pakistan, hence these countries adopted vaccination at either national or local level for prevention from H9N2.[5,40] It is needed to understand the molecular determinant of H9N2 antigenicity, genetic drift, zoonotic potential, and viral fitness consequences for making a next generation vaccine against H9N2 virus. Stamping out by culling of influenza-infected birds was used as a first line of defense in countries without a history of H9N2 virus infection.[41,42] Attenuated cold-adapted H9N2 influenza virus vaccine selected to control the influenza virus infection in chickens.[43]

Some other interventions have been used in the field to reduce or stop the spread of influenza virus infection in poultry. Live bird markets are the main hot spot to spread the infection due to availability of different species of poultry from across wide range of geographical area. Hence, different interventions including biosecurity, temporary/periodic closures of markets and hygienic conditions were used to control the infection. There are currently no clear methods for treating H9N2 avian influenza. To control the pandemic potential and human-to-human transmission of H9N2 globally, biosecurity in the poultry industry, isolation of infected poultry, decrease in microbial infection, screening programs for high-risk areas and decreasing the opportunities for reassortment are practical requirements.[44]

CONCLUSION

In last decade, the outbreak of H9N2 has increased worldwide in different geographical ranges, causing damage to poultry production and great economic loss to poultry industries. Mostly, H9N2 virus causes low mortality and mild disease but recently increasing infections in humans and animals show that these viruses may cause pandemic infection in the near future. Due to the presence of human receptor binding protein in some H9N2 subtype which allow transmission between humans, and presence of internal gene cassettes in some other H9N2 subtypes, which allow efficient replication in humans, these intersubtypic H9N2 virus could cause a pandemic due to the zoonotic potential of these viruses. Hence, there is urgent need of a specific vaccine against these viruses and surveillance efforts are also required in infected areas of poultry.

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Conflicts of interest
There are no conflicts of interest.

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