

# H9N2 low pathogenic avian influenza in India – a simmering inferno?

Quantum growth exhibited by the Indian poultry industry in the last four decades is phenomenal (Fig. 1). With annual production of 82,000 million eggs and 4.2 million tons of poultry meat in 2016, India is now among the biggest egg and broiler producers in the world.

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At the current level of production, the poultry sector contributes about USD\$10.0 billion to the gross national product and provides direct and indirect employment to over 3.5 million people. This growth has been driven by ever increasing demand from the growing population, urban migration, rising incomes and choice conversions.

To some extent improvements in infrastructure, vertical integrations, availability of new technologies and efficacious vaccines have further supported this growth.

However, despite this fast paced growth, India still lags behind several countries in terms of overall egg and meat production, per-capita availability and it is miles to go before she can compare with the developed world.

The poultry industry in India struggles with several problems to sustain the growth momentum, not only to meet the growing domestic demand but also to remain competitive in order to bear the brunt of the import of cheaper eggs and meat in the times to come.

There have been, and still are, several economic and situational roadblocks on the nutritional, infrastructure, and technology fronts and not least, infectious diseases, amongst which avian influenza has played a major role in the last decade.

A bird flu incidence, besides causing direct losses, also triggers a panic situation among egg and poultry meat consumers in India resulting in a significant drop in demand causing instability in a sensitive and

volatile market, thus leading to irrecoverable losses and consequent growth retardation.

Although outbreaks of Highly Pathogenic Avian Influenza (HPAI) due to H5N1 (and now H5N8) viruses occur sporadically causing losses in the affected area, it is the Low Pathogenic Avian Influenza that has become endemic and runs in the background causing severe economic loss to the industry.

This article focuses on implications of (H9N2 LPAI) for the Indian poultry industry and begs the question, “Why should we not take appropriate action (read vaccinate) for prevention of this economically devastating disease?”

We need to act not only to protect the poultry industry but also the human population from this WHO acknowledged zoonotic possibility which simmers in the background waiting to turn into an inferno.

## Avian influenza in brief

Influenza is caused by orthomyxoviruses belonging to Type A influenza virus group of the family of six known genera (influenza A, B and C) and togogovirus (human), isavirus (fish) and quaranavirus (human).

Influenza virus type A is an enveloped virus with a negative-sense, single-stranded RNA genome organised in eight gene segments, which encode at least 11 proteins.

Antigenic and genetic diversity of two surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), is used to classify type A influenza viruses into subtypes; 18 HA and 11 NA subtypes are known to date.

Water- and shorebirds were identified as reservoirs harbouring all subtypes, except A(H17N10) and A(H18N11) of which RNA was recently detected in bats from Guatemala and Peru respectively. Reservoir animals typically do not display symptoms.

In contrast, the diversity of influenza viruses in mammalian hosts is limited to specific subtypes of Influenza Type A virus. Human-

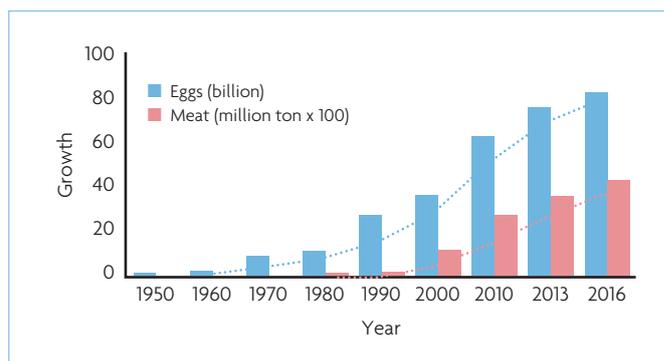


Fig. 1. Growth of poultry in India 1950-2016 (Various sources – FAO, GAIN and DAHFD GoI).

adapted seasonal influenza viruses since the beginning of the 20th Century have had HA subtypes H1, H2 and H3, combined with NA subtypes N1 and N2. Avian adapted viruses are mostly observed in H5, H7 and H9 subtypes.

Based on the severity of the disease, avian influenza viruses are further designated as Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenic Avian Influenza (LPAI). These are differentiated by pathogenicity tests in chickens and by molecular differentiation of HA gene characteristics.

## LPAI

LPAI is universally present in poultry growing countries involving a multitude of H- and N- subtypes with varying degrees of pathogenicity, prominent among these have been those due to H5, H7 and H9 subtypes. The H5 and H7 viruses have been shown to share genes with H9 viruses occasionally to evolve to HPAI in some instances.

HPAI due to H9N2 by itself has not been reported so far. LPAI outbreaks due to H5 or H7 subtypes are notifiable (LPNAI), whereas those due to H9 are not. The H9N2 virus causes mild to severe respiratory disease but exposes and debilitates the chicken, which then succumbs to secondary infections leading to high mortality and egg production losses. H9N2 virus is generally regarded as a silent operator

contributing genes for enhancement of virulence of other avian influenza viruses and also enabling them to infect mammalian species.

## The global situation

Probably the oldest report of H9N2 LPAI was recorded in the USA in 1966 with the isolation of AIV from turkeys showing respiratory disease. Thereafter, H9N2 has been reported from all the continents of the world.

In domestic avian species in North America, H9N2 influenza viruses occur primarily in turkeys, occasionally in quails, and rarely, if ever, in chickens.

In Asia, long term surveillance in live poultry markets in Hong Kong from 1975 to 1985 detected H9N2 influenza viruses in apparently healthy ducks but not in chickens.

The virus then seems to have adapted to chickens and since the early 1990s, H9N2 influenza viruses have become widespread in domestic chickens in Asia.

Molecular characterisation and phylogenetic analysis has shown that H9N2 AIVs circulate in multiple lineages around the world.

Two distinct lineages of H9N2 influenza viruses have been identified – North American and Eurasian. The Eurasian lineage can further be subdivided into three major sublineages – the G1 (BG94, G9), Y280 (Y439) and Korean.

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Most of the H9N2 viruses cause a mild infection. Those of the North American lineage have not been reported to cause a significant disease although have been considered to be originators of Eurasian lineages and are not considered important for poultry at present.

Among the Eurasian viruses the H9N2 of G1 lineages have been most prevalent in South Asia.

There are several incidences of the Eurasian LPAI viruses of G1/G9 lineage causing clinically significant infection resulting in high morbidity and mortality in affected chicken flocks.

### **Incidence of H9N2 LPAI in Asian countries**

#### ● **China**

The first outbreak of the H9N2 influenza virus in China occurred in Guangdong province of Southern China during November 1992 to May 1994 affecting 17 chicken farms, including broilers and layers.

In broilers 10-40% mortality was noticed, while there was a 14-75% drop in egg production. Following this outbreak, the H9N2 infection sporadically occurred in chickens, ducks, and geese and spread to most provinces of China by 1998, and is now the most prevalent subtype of influenza viruses in chickens in China.

The H9N2 virus now co-circulates with H5N1 in south eastern poultry populations and evidence suggests that it plays an important role in the emergence of reassortants with potential to infect mammals.

Human cases with H9N2 virus have also been recorded in Hong Kong in 1999 and 2003.

China recommended vaccination against H9N2 LPAI since 1998 and currently 20 different vaccines are produced and applied in China.

#### ● **Israel**

In Israel, H9N2 outbreaks were first recorded in 2000 at chicken and turkey farms, followed by several occurrences in later years.

The viruses were phylogenetically analysed to belong to G1 Eurasian lineage. Initially, autovaccines were applied but later commercially produced vaccines were recommended from 2002. Although virus has not been eliminated from poultry and turkey populations, economic losses have been significantly reduced by vaccinating the flocks.

#### ● **Korea**

South Korea experienced LPAI of economic significance in 1996 and since 2000 it has been accepted as an endemic infection in the poultry population of the country.

Initially, a stamping-out and compensation policy was pursued but later in 2004 the Korean authorities permitted the use of inactivated H9N2 vaccines.

The Committee on the National AI Vaccine Campaign determined that using a single vaccine strain was the most effective strategy with which to resolve the H9N2 AI situation in Korea.

The importance of continuous serological and molecular monitoring was stressed for proper vaccine strain selection to remain ahead of rapidly evolving H9N2 viruses.

#### ● **Egypt**

In Egypt, the sero-prevalence of H9N2 antibodies in chicken populations was reported in 2001 and the first isolation report of H9N2 virus

was in 2010-2011 in chickens, quails and turkeys. Kandeil et al (2014) studied 22 Egyptian H9N2 isolates from 2011 to 2013 and observed that multiple mammalian-host-associated mutations were detected that favour transmission from avian to mammalian hosts.

Phylogenetic data showed that Egyptian H9N2 viruses were closely related to viruses isolated from neighbouring Middle Eastern countries, and their HA gene resembled those of viruses of the G1-like lineage. A human case of confirmed H9N2 influenza was reported in Aswan Governorate in a three year old male in 2015.

Vaccination for the prevention of H9N2 infection has been implemented in Egypt since 2014.

#### ● **Iran**

H9N2 subtype influenza A virus of low pathogenicity was reported in Iran in 1998. Although the virus was identified as of low pathogenicity it caused mortality of up to 68% in some broiler farms.

Vaccination of chickens with inactivated vaccine was recommended to prevent the infection from H9N2 LPAI in 2002. Despite extensive application of inactivated vaccine against H9N2 subtype, the H9N2 virus is circulating in Iranian chicken flocks causing notable mortality, particularly in broiler chickens. The authors observed that proper phylogenetic analysis needs to be carried out for selection of a vaccine strain to match antigenicity of contemporary viruses for the success of vaccination campaigns.

To summarise, it is evident that economic losses suffered by the poultry industry have prompted China, Korea, Iran, Israel and Egypt

to take the prevalence of the H9N2 virus seriously and concerted efforts have been made to reduce the incidence and virus load through vaccination. Although it has not been possible to prevent circulation of H9N2 viruses, losses due to primary infection and secondary complications have been reduced. It is generally believed that if proper vaccine strains are selected for the given geographical region better results can be expected.

#### ● **Pakistan and Bangladesh**

H9N2 viruses have also been reported in countries sharing a porous border with India.

Pakistan and Bangladesh have both reported incidences of H9N2 LPAI which have caused disease and losses in poultry farms in both countries and the H9 viruses from both countries showed close relation to G1 lineage.

Human cases of H9N2 infection have been reported from Bangladesh in 2011 and 2015.

#### ● **India**

Most likely, AI in India has been known to exist as a silent infection for a long time. A duck influenza virus strain H4N2 was isolated from the cloacal swab of a domestic duck from Tirunelveli town, Tamil Nadu in 1978. Duck influenza virus strains H9N2 and H9N3 were isolated during an outbreak of respiratory distress with 15-20% mortality at a duck farm in Kerala in 1985.

Many researchers have reported isolation of H9N2 viruses from poultry farms across India.

LPAI as an overt, loss causing, clinical disease due to H9N2 surfaced in 2003 in India. Infection and isolation of virus from domestic poultry was reported in 2003-04 during investi-

gations in poultry farms with a history of reduced egg production and respiratory illness in several states of India. H9N2 virus was consistently isolated from these outbreaks.

Similar cases of respiratory illness were reported from layer poultry farms in Gujarat state of India with up to 30% mortality.

Clinical disease signs and gross pathology at necropsy typical of avian flu were observed followed by virus isolation and characterisation. Affected flocks showed illness for 3-4 weeks with a drop in egg production of up to 40%.

Approximately 2-3% and 10-30% of mortality was observed in affected grower and layer flocks, respectively. At present it is adequately established that H9N2 LPAI is endemic in India.

Nagarajan et al, 2009 reported pathotyping of six representative isolates by intravenous pathogenicity index (0.0/3.0) in 6-8 week old chickens, trypsin dependency in cell culture and HA cleavage site analysis. They confirmed that these isolates were of low pathogenicity.

Nucleotide sequence analysis of the HA gene showed that the Indian isolates were very closely related (95.0-99.6%) and shared a homology of 92-96% with H9N2 isolates from Germany and Asian regions other than that of mainland China.

The authors concluded that the H9N2 viruses studied shared a common ancestor Qa/HK/G1/97 isolate which had contributed internal genes of H5N1 virus circulating in Vietnam, indicating that H9N2 in India could have evolved from both Eurasian and Chinese lineages.

Dash et al 2014, in their pathogenicity studies, reported that although experimental infection of chickens with their H9N2 isolate (IVPI 0.0/3.0) produced no clinical disease, the same virus in farm chickens had caused serious clinical disease and mortality, mostly due to secondary infections.

Pawar et al (2012) isolated H9N2 and H4N6 viruses from migratory birds and domestic ducks and poultry in West Bengal and observed that antibodies against AI H5 and H7 in ducks indicated the probable role of domestic ducks in the transmission of AI viruses.

With the evidence of many isolations of H9N2 from 2003 until date it can be concluded that H9N2 is widely prevalent across the Indian subcontinent including Pakistan and Bangladesh.

Human cases in Bangladesh also emphasise the need to address H9N2 prevalence from the point of view of preventing the disease in poultry by vaccination.

H5N1 HPAI outbreaks have frequently been encountered in these three countries, which are also on migratory bird routes, have

live bird markets, and thus provide favourable conditions for transition of AIVs from migratory birds to water fowl to poultry and for coexistence of H9N2 and H5N1 with its consequences.

### Potential threats of H9N2 LPAI prevalence

#### ● H9N2 LPAI – Apparently insignificant but a potential threat to poultry and humans.

From the emergence of H9N2 LPAI as an economically important disease in the 1990s to the current times across Asia, it has been amply demonstrated that these viruses circulate in the poultry in live bird markets in these countries and persist in the comfort of being apparently innocuous as they have 0.0/3.0 IVPI and cannot be demonstrated to cause disease under experimental conditions.

For this reason alone, they are not considered primary pathogens and have not been listed as notifiable diseases by the OIE.

However, serological and phylogenetic studies have demonstrated that these have been instrumental in delivering avian HPAs of H5N-, and H7N- subtypes and have themselves infected humans (China, Bangladesh) over the last three decades. Therefore, these must be given due attention and significance in AI prevention and control strategies in international fora.

#### ● H9N2 may itself evolve into a pandemic influenza agent.

Work done so far by various scientists indicates that the H9N2 class of Influenza A viruses have distinctively evolved in the LPAI group as AI viruses that can infect not only avian species but also humans and swine. Isolation from multiple host species combined with its coexistence with other type A influenza viruses has resulted in accumulated mutations and generation of novel variants, thus giving H9N2 a potential capability to evolve into a pandemic virus.

#### ● Co-circulation with H5N1 can lead to enhancement of H5N1 virulence for poultry as well as humans.

LPAI viruses of subtype H9N2 co-circulated simultaneously with HPAI viruses of subtype H5N1 in commercial and backyard poultry in Bangladesh. The authors reported a close relationship LPAI H9N2 from Bangladesh to Indian, Pakistani and Middle Eastern isolates and identified an ancestor relationship G1 lineage.

The internal genes showed high sequence homology to an HPAI virus of subtype H7N3 from Pakistan, whereas the PB1 gene showed similarly high nucleotide

homologies to recently circulating HPAI H5N1 from Bangladesh, revealing two independent reassortment events providing evidence of gene sharing between H9N2 and other LPAI viruses.

Phylogenetic analysis showed that H7N9 and H10N8 viruses possess internal gene cassettes recruited from poultry H9N2 virus. Long-term surveillance of poultry in China and east Asia shows that H9N2 virus is widely spread.

Phylogenetics analyses suggest that different subtypes of avian influenza viruses cocirculated with H9N2 and have reassorted their internal genes.

#### ● H9N2 can re-assort with viruses from migratory birds leading to newer mutants in the domestic water fowl which may then co-infect poultry with LPAI viruses to generate new strains.

Several authors have demonstrated that water fowl and ducks can pick up AI viruses from migratory birds and if H9N2 infected chickens are in close contact with these ducks, a reassortant virus can evolve and HPAI due to H7N- or H5N- can be the result. Since H9N2 viruses themselves have the potential to infect humans, their coinfection in poultry with H5N1 viruses can give rise to a human capable H5N1 virus with pandemic potential, as suspected with regard to human infections in Hong Kong in 1997.

#### ● H9N2 may itself evolve to be HPAI by selection of escape mutants in partially immune poultry populations.

Given the situation in Asian countries where the live bird market is an unavoidable phenomena, and H9N2 viruses have been in circulation for several years, it is perceptible that a partially immune poultry population exists which favours the selection of escape mutants towards higher pathogenicity.

This may be the reason that once regarded as a non-significant infection in poultry, the H9N2 LPAI has surfaced as an economically threatening disease in the last 15 years with an increasing number of countries having to resort to vaccination for prevention of LPAI alongside HPAI infections.

### Current situation and concerns

The prevalence of H9N2 viruses has been established beyond doubt in the Indian poultry population. Given the fact that live bird markets are a reality in India too, the virus can be assumed to circulate, mutate and reassort freely.

Widespread seroprevalence also indicates unabated circulation of this virus throughout the sub-

continent with frequent episodes of infection, secondary complication and consequent heavy losses.

Recurring outbreaks with H5N1 year after year since 2006 (and now with H5N8 in 2016) must acknowledge the fact that when H9N2 and H5N1 co-circulate the reassortants can exhibit undesirable results.

As is evident from reviews of published research, India, like other Middle Eastern and South Asian nations must also take cognisance of the threat posed by the ubiquitous presence of H9N2 AI virus and initiate necessary efforts and action to arrest the environmental load and evolution of H9N2 in order to prevent the emergence of HPAI viruses of higher pathogenicity and the emergence of a human H9N2 in the future.

In the absence of a policy of vaccination against H5N1 HPAI, it becomes all the more important to reduce and eliminate H9N2 virus from the scene.

The invaluable indigenous breeding stock germ plasm comprising the pure lines and grandparent stocks developed and improved over the last six decades may be wiped out in a short time if H5N1 strikes in the want of a clear cut policy.

Certainly we do not want to practice stamping out in such stock which could spell the doom for our poultry industry.

Although not hitherto reported from humans in India (probably because only well cooked chicken meat is consumed in this country), evidence from Bangladesh suggests that the people handling sick birds infected with H9N2 can also pick up the virus and become diseased.

This indicates that a possibility always exists that the avian flu viruses of 'happy-to-share-genes' H9N2 class may eventually turn up a human infecting reassortant in India too.

Therefore, having established that H9N2 LPAI is a major hurdle in sustaining the growth of the poultry industry we propose that there is an urgent need to acknowledge the problem of H9N2 avian influenza on the subcontinent and appoint a task force to:

- Study in detail the molecular characteristics of prevalent H9N2 viruses across the country.
- Select an appropriate candidate vaccine strain based on molecular cartography, serological and animal challenge methods.
- Recommend a policy of vaccination against LPAI.
- Invite vaccine manufacturers to produce a properly regulated high quality, region-specific vaccine if required. ■

References are available from the author on request