

Prevention and Control

Anti viral drugs against LPAI is not being recommended for use in poultry. Mortality can be kept under control by taking care of secondary invaders like *E.coli* and *Mycoplasma* with suitable drugs. *E.coli* resistance to almost all available antibiotics and antibacterial for poultry use is a major obstacle in bringing mortality under control particularly in broilers. Use of bronchodilators and mucin liquefying agents for opening the air ways help in reducing mortality. Use of water sanitizers and disinfectant spray during phase of outbreak helps in reducing viral and bacterial load. Additional vitamins and trace minerals should be added in the feed to compensate low intake due to reduced feed intake during the outbreak.

Disease can be prevented by use of good quality inactivated (killed) oil adjuvant vaccine. Live vaccine is not used because of fear of mutation. Inactivated H9N2 vaccine has some limitations in providing good protective titers. These are

1. Meat type birds-broilers- respond less compared to white leghorn birds in developing titers. **Light breeds produce good levels of antibodies (5-7HI Titers) even after a single application, while heavy breeders have a weaker response requiring at least 2-3 applications of the vaccine in order to achieve protective titers of antibodies.**
2. As there is no priming with live vaccine virus, local protective Ig A antibodies are not produced in the respiratory tract which is the first site of entry of the virus.
3. Circulating neutralizing antibodies do not provide enough protection at the respiratory epithelial level. **However, circulating high level Ig G (HI 5-7 and more) induced by killed vaccine may in some way reach the tracheal epithelium and reduce the colonization and multiplication of H9N2 virus.**

Use of inactivated H9N2 vaccine in commercial layers has provided good protection. Vaccinating layer chicks with half dose at 7th day and full dose at prelay and mid lay stage has shown satisfactory results. **Breeders/commercial layers can be vaccinated at 12-15 days, a second vaccination at 6-10 weeks of age and a third vaccination at 18 weeks of age to get the antibody titers of 5-7.** This schedule can be modified based on the experience and the virus challenges in an endemic area. Protection level achieved in the commercial broiler chicks after vaccination during first 5 days of age is doubtful and often frustrating. **Vaccination with full dose at 12-14 days and repeated at 24th day assures good protection provided the birds do not get exposed before 28 days of age. This is generally not happening in the field due to poor biosecurity level.**

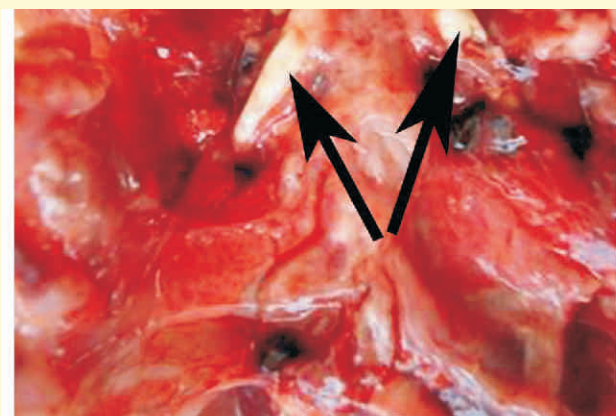


Fig.6. Bronchial plugs in LPAI H9N2

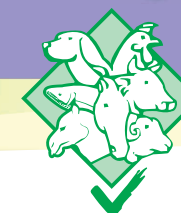
A different approach is therefore needed for commercial broilers to reduce losses with or without use of killed vaccine. This includes

1. Procurement of chicks with minimum mycoplasma load.
2. Chicks with higher MAb against H9N2 perform better. This can be achieved through repeated killed vaccinations in breeders.
3. Day old Marek's vaccination helps in maintaining good CMI as all broiler chicks are exposed to field Marek's disease virus from day one age. MD pathogenesis progresses along with growth of the birds but mortality is not seen because of longer incubation period and short commercial life of the bird. Such birds do exhibit suboptimal CMI response.
4. Use of lentogenic ND vaccine strains causing low respiratory reactions.
5. Avoid rolling of vaccine virus by proper method of drinking water vaccination.
6. Use of anti mycoplasmal preventing programme
7. Biosecurity should be practiced in its true sense.

LPAI H9N2 and HPAI H5N1 have been widespread in poultry across large areas of the world resulting in a modified eco-epidemiology and a zoonotic potential. In recent years the H9N2 viruses have undergone extensive genetic reassortment which has led to the generation of H9N2 viruses of novel genotypes in the Indian sub continent. The novel genotypes of H9N2 viruses may play a role in the increased problems observed by H9N2 to poultry and reinforce the continued need to monitor H9N2 infection for their zoonotic potential also. There are few reports that it has caused mild non fatal conjunctivitis in man. However, no such reports from India. For H5N1 there are mandatory strict control measures implemented by the authority and hence it has remained under control. But for H9N2 being a LPAI virus there are no such control measures. This allows the virus to spread widely and become endemic. An extraordinary effort is required to manage these epidemics from both the human and poultry health perspectives.



VARSHA VAHINI



From the Editor's Desk

We Wish All readers A Happy New Year

We have a pleasure in bringing out the first edition of the New Year. This issue covers the most prevalent and frequently talked about disease LPAI. We are happy to present to you a detailed account on the same from Dr K.S.Prajapati, Professor & Head, Department of Pathology, Veterinary College, Anand, Gujarat.who is continuously engaged in educating farmers on LPAI with his rich experience.

We thankfully acknowledge his valuable contribution. We are sure this would provide a practical insight on the disease and its control.

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LOW PATHOGENIC AVIAN INFLUENZA H9N2 INFECTION IN CHICKEN

(So called variant/VVND)

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Introduction

Influenza viruses are enveloped, segmented, single stranded, negative sense RNA viruses of the family *Orthomyxoviridae*, and are divided into types A, B, and C. Type A is seen in man, birds, pig, horses and many other animals while B and C are only seen in man. Influenza A viruses isolated from birds are known as avian influenza viruses (AIV) and are further divided into 16 haemagglutinin (HA) and 9 neuraminidase (NA) subtypes. AIV vary in their ability to produce infection, disease and death in different bird species. Based on the pathobiological effects in chickens, AIV are categorized as low pathogenic avian influenza (LPAI) virus or highly pathogenic avian influenza (HPAI) virus. The fear caused by the HPAI viruses has obscured the importance of the much larger and more common group of LPAI viruses. LPAI viruses by definition are unable to cause death in SPF chickens under laboratory conditions. However, under

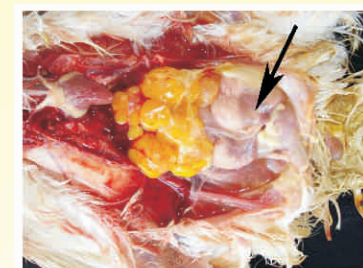


Fig.1. Oviduct wall edema

field situations it terminates into low to heavy mortality and production drops with clinical signs and lesions of respiratory, digestive and reproductive systems causing severe economic losses to the poultry farmers.

Among various LPAI viruses sub type H9N2 has become the most important infection throughout the world and is globally prevalent in domestic poultry since 1990s and has reached panzootic proportions. The natural avian reservoir of H9 viruses are shorebirds and gulls. Outbreaks of LPAI H9N2 occurred in domestic ducks, chickens and turkeys in Germany during 1995 to 1998, in chickens in Italy in 1994 and 1996, Pheasants in Ireland in 1997, ostriches in South Africa in 1995 and turkeys in the US in 1995 and 1996. Wide spread infections due to H9N2 in chicken were reported in China and Hong Kong during 1994 and Korea in 1996. LPAI H9N2 infections have also been reported in the Middle East since



1998 and have also caused wide spread out breaks in commercial chickens in Iran and Pakistan often associated with serious disease problems. The impact of avian influenza caused by H9N2 viruses in Pakistan is now significantly more severe than in previous years. Iqbal *et al.* (2009) identified novel genotype of H9N2 influenza A viruses isolated from poultry in Pakistan containing NS genes similar to highly pathogenic H7N3 and H5N1 viruses.

The disease is prevailing in India since last seven years and is popularly known as variant or VVND. It is wide spread and has become endemic in most part of the country among commercial broilers, commercial layers and breeders. For broiler farmers it has become the major cause of concern as economic viability of farming is affected. In spite of being low pathogenic virus its ability to cause high mortality is attributed to secondary complications with *E.coli*, mycoplasma, infectious bronchitis virus and New castle disease virus. In the initial years the out breaks were mostly seasonal. It used to start during the end of winter (Feb.-March) and disappear at the beginning of monsoon. However, in recent years the seasonal pattern has disappeared and has become perennial in nature.

Pathogenesis

H9N2 viruses multiply in respiratory epithelium, digestive epithelium and have also affinity for oviduct epithelium. The disease spread through contact, droplet infection or through ingestion of contaminated feed and water. Long distance spread is less likely to occur as the virus is highly fragile. However, it remains infective in wet litter for many days. Increased mortality due to H9N2 infection in the field situation is explained by its unique modulatory effect on immune system. In the influenza virus infected host macrophages become infected after the primary target respiratory ciliated epithelial cells. How macrophages function after influenza virus infection is critical in the host's immune response to the virus and thus to viral pathogenesis. The H9N2 NS1 protein is a potent modulator of the host immune responses in chicken macrophages and respiratory epithelial cells. NS1 suppressed the apoptic (programmed cell death) process through suppression of the induction of FasL allowing sufficient time for replication of the virus. Though pro inflammatory cytokines are largely up regulated, the interferon (first line of defense) response is fairly weak. This allows uncontrolled proliferation of virus attacking many respiratory epithelial cells. MHC antigens II and IL-4 are down regulated which are pivotal in the activation of CD4+ helper T cells and humoral immunity. In H9N2 infected chicken the neutralizing antibodies and humoral immunity may not develop efficiently which is a unique feature of this sub types as compared to other LPAI viruses. End result of this adverse immune modulation is that once the bird is infected the virus remains in the respiratory system for longer periods providing opportunities for the already

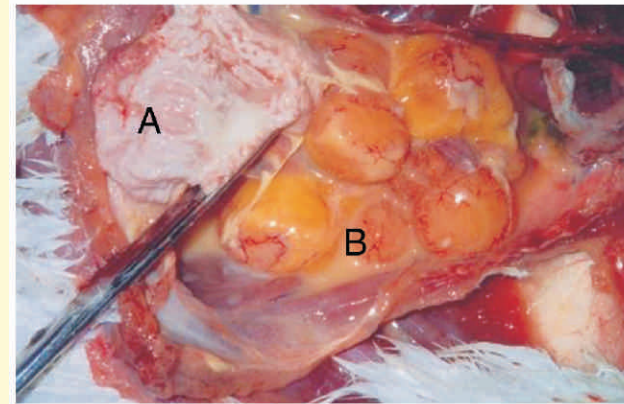


Fig.2. Excess free albumin in the oviduct (A) and watery yellow fluid (B) in the peritoneal cavity

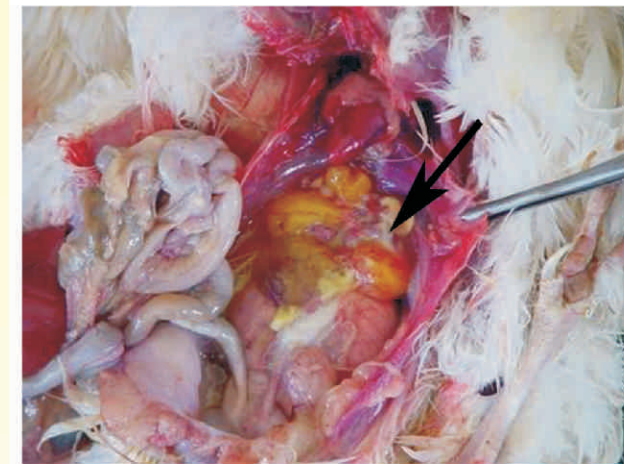


Fig.3. Fibrin flacks over the ovary and in the abdominal air sac.

available pathogen around the birds like *E.coli*, Mycoplasma mainly and IBV and NDV occasionally to attack and complicate the disease leading to heavy mortality and production drops.

Commercial layers

A. Clinical signs:

1. All age group flocks are susceptible. However, it is more frequent during laying phase than growing phase. This is because of the peculiarity of H9N2 to have additional affinity for functional oviduct.
2. The onset is sudden with depression, dullness and ruffled feathers.
3. Head swelling and respiratory rales are mild and not prominent as in case of broilers.
4. Feed consumption is reduced by 15-20 % and continues for 8-10 days.
5. Production drop is slow and reaches 30-40% drop within two weeks in non vaccinated flock.



6. Production starts recovering after 2-3 weeks but fails to reach original production before 5 weeks. Returning of the flock to original production depends on the magnitude of the drop.
7. Egg shell quality and the internal quality of eggs are unaffected except few lathery eggs in the initial period of production drops.
8. Egg size is reduced because of low feed intake.
9. Mortality in non vaccinated flock is around 8-10 % but sometimes exceed 25%. Mortality usually runs for 8-10 days.
10. In vaccinated flocks mortality is generally not seen but may cause transient production drops of 3-10 % depending on immunity status.

B. Post mortem lesions

1. Postmortem lesions are mainly observed in respiratory, genital and digestive system.
2. Mild head swelling, conjunctivitis and excess nasal mucous with fibrin clots.
3. Mild to severe congestion sometimes leading to hemorrhages is seen in trachea along with excess mucus. White fibrin flacks like material is often found

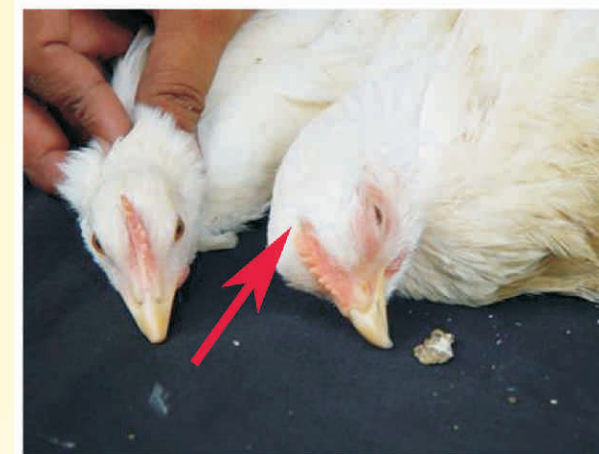


Fig.4. Swollen head in LPAI H9N2 infection

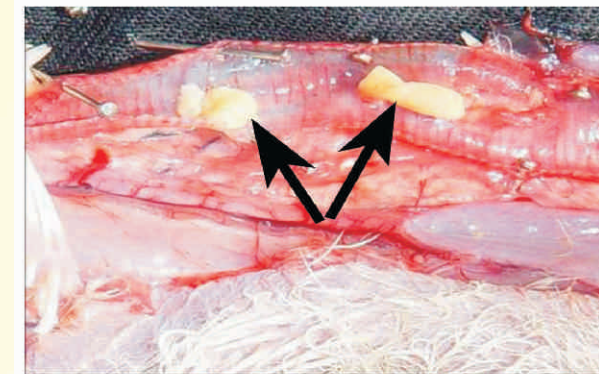


Fig.5. Caseous mass in the trachea.



- in the lumen.
4. Lung shows congestion and edema in most cases. Few cases show consolidation at the insertion of bronchi.
5. Oviduct wall is highly edematous (Fig.1). This lesion has diagnostic significance. Oviduct lumen is filled with clear excessive egg albumin while the abdominal cavity contains relatively large quantity of thick, white-yellow colored fluid (Fig.2).
6. Thoracic and abdominal air sacs are edematous, frothy and contained fibrinous exudates. Ovarian follicles are covered with fibrin flacks (Fig.3).
7. Proventriculus shows petechial hemorrhages.
8. Pancreas is enlarged and hardened in few cases.
9. Other organ like intestine, heart, liver, kidney and spleen do not show significant gross lesions.

Commercial broilers

A. Clinical signs:

1. Out breaks in commercial broilers are more frequent and more severe as meat type birds are more susceptible than egg type birds.
2. Beginning of third week or later the chicks start sneezing which spreads fast in the flock.
3. After 4-5 days the respiratory rales become more prominent and mortality starts with secondary complications of *E.coli*.
4. Facial edema or head swelling is more prominent (Fig.4).
5. Feed intake is static or reduced.
6. Weight gain is also static or reduced.
7. Mortality ranges from 4 % to 60% even in antibiotic treated flocks.
8. Flock becomes uneven and feed efficiency is reduced.

B. Post mortem lesions:

1. Post mortem lesions in respiratory system are more severe compared to layers.
2. Head swelling is due to S/C edema and cellulitis.
3. Trachea is severely congested with excessive mucus and caseative material in the lumen (Fig.5).
4. In most cases fibrinonecrotic tubular casts is noticed in one or both the bronchi at tracheal bifurcation (Fig.6). This lesion has diagnostic significance. Such birds die due to suffocation and often have no lesions of pericarditis and perihepatitis.
5. Lungs show congestion, edema and haemorrhages.
6. Thoracic and abdominal air sacs are thickened, edematous, and cloudy and often show deposition of variable amount of cheesy material.
7. As the outbreaks are always complicated with secondary *E.coli* infection, fibrinous pericarditis – perihepatitis, suppurative pleuritis and caseative salpingitis are also seen.