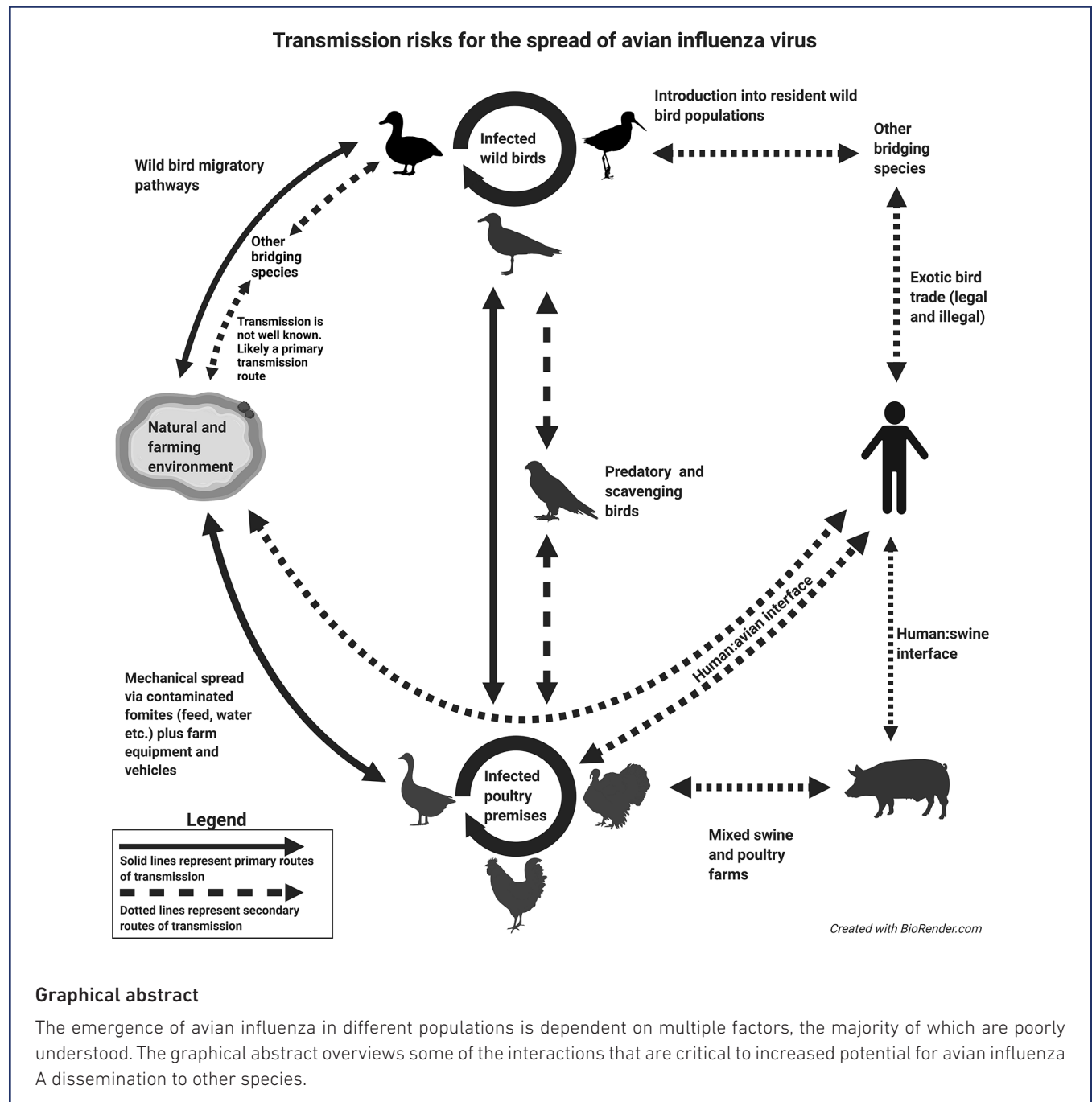


JMM Profile: Avian influenza: a veterinary pathogen with zoonotic potential

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Abstract

Avian influenza viruses (AIVs) are classified as either low pathogenicity (LP; generally causing sub-clinical to mild infections) or high pathogenicity (HP; capable of causing significant mortality events in birds). To date, HPAIVs appear to be restricted to the haemagglutinin (HA) glycoprotein H5 and H7 AIV subtypes. Both LPAIV and HPAIV H5 and H7 AIV subtypes are classified as the causative agents of notifiable disease in poultry. A broad range of non-H5/non-H7 LPAIVs also exist that have been associated with more severe disease outcomes in avian species. As a result, the constant threat from AIVs causes significant economic damage in poultry production systems worldwide. The close proximity between mammalian and susceptible avian species in some environments provides the opportunity for both inter-host transmission and mammalian adaptation, potentially resulting in novel AIV strains capable of infecting humans.

HISTORICAL PERSPECTIVE

The earliest avian influenza virus (AIV) historical record dates from 1878, in Perroncito's description of a highly contagious disease affecting domestic fowl in Italy. This disease would later be known as fowl plague, and then as avian influenza [1].

CLINICAL PRESENTATION

Clinical presentation is variable, depending on both virus pathogenicity and avian host species. Clinical signs include the following.

- (i) Low pathogenicity avian influenza viruses (LPAIVs): sub-clinical, mild respiratory/enteric disease, decreased egg production and low (if any) mortality, although moderate to high mortalities have also been reported for LPAIVs.
- (ii) High-pathogenicity avian influenza viruses (HPAIVs): severe acute systemic disease with sudden-onset high flock mortality in terrestrial poultry. Cessation of egg laying, respiratory signs, cyanosis, oedema, ischaemic necrosis of combs and wattles; subcutaneous haemorrhage and profuse diarrhoea, plus a range of neurological impairments in severe cases.

MICROBIAL CHARACTERISTICS: PHENOTYPIC AND GENOTYPIC FEATURES

AIVs are enveloped, negative sense, single-stranded RNA viruses classified within the genus *Alphainfluenzavirus*, family *Orthomyxoviridae*. Genomes contain 8 segments of viral RNA, totalling ~13500 nucleotides that encode at least 10 'core' proteins, with some strains encoding up to 18 different proteins by utilizing alternate reading frames. The core influenza proteome includes: polymerase basic protein 2 (PB2), polymerase basic protein 1 (PB1), polymerase acidic protein (PA), haemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix 1 (M1) and matrix 2 (M2), non-structural protein 1 (NS1) and non-structural protein 2 (NS2, or nuclear export protein [NEP]). Virions derive their envelope from the host cell as they bud during virus egress. Virions can be spherical, typically ranging from 80 to 120 nm in diameter, or filamentous.

The latter form can extend to many tens of micrometres in length and is generally reported in clinical samples, whilst laboratory isolates are often pleiomorphic in shape [2].

Importantly, AIVs exhibit genetic and antigenic diversity through two key mechanisms. Antigenic drift is the process whereby genetic mutations (incorporated by error-prone viral polymerase activity) affect the external glycoproteins (HA and NA). AIV HA and NA subtypes are defined as H1–H16 and N1–N9 subtypes, respectively [2]. Antigenic drift results from cumulative mutations that may alter the phenotypic and antigenic profile of the virus, alongside genetic changes within the remaining gene segments. Antigenic shift describes an abrupt alteration in viral phenotype and antigenicity brought about by genetic reassortment of the glycoproteins following coinfection of the same cell. Antigenic shift can lead to the emergence of a virus with a completely novel antigenic and phenotypic profile.

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Abbreviations: AIV, avian influenza virus; CS, cleavage site; EFE, embryonated fowls' eggs; FAO, Food and Agriculture Organization; GsGd, goose Guangdong; HA, haemagglutinin; HI, haemagglutination inhibition; HPAIV, high pathogenicity avian influenza virus; IAV, influenza A virus; LPAIV, low pathogenicity avian influenza virus; NA, neuraminidase; OIE, World Organisation for Animal Health; PCR, polymerase chain reaction; rRT-PCR, reverse transcription real-time PCR; VI, virus isolation; WHO, World Health Organisation.

CLINICAL DIAGNOSIS, LABORATORY CONFIRMATION AND SAFETY

Clinical diagnosis

There are no pathognomonic clinical signs for AIV infection. LPAIVs typically cause mild respiratory signs (e.g. snicking) and/or production parameter changes (e.g. reduction in egg production and weight gain, etc.), and can be clinically indistinguishable from other poultry respiratory and enteric diseases, such as manifestations of infectious bronchitis, laryngotracheitis, avian orthoavulavirus and paramyxovirus infections. For HPAIVs, differential diagnoses for sudden flock mortality (in galliformes) and more aggressive manifestations of the above clinical signs also feature in virulent Newcastle disease and septicaemic fowl cholera.

Laboratory confirmation

Diagnosis relies on submission of clinical material (swabs and tissues) for laboratory investigation. Molecular testing by rigorously validated reverse transcription real-time (rRT)-PCR assays targeting conserved genes (such as the M1 and/or NP) is the frontline diagnostic method in many laboratories, along with H5 and H7 subtype-specific rRT-PCRs to detect the notifiable diseases [3]. Secondary diagnosis involves virus isolation (VI) using embryonated fowls' eggs (EFEs) with classical subtyping by haemagglutination inhibition (HI) with defined antisera [4]. HPAIV is confirmed where multi-basic amino acid changes in the HA cleavage site (CS) are detected by PCR and sequencing and/or by an OIE confirmatory intravenous pathogenicity index (IVPI) score >1.2 (range 0–3.0) [4]. AIV subtype-specific antibodies to the HA are detected by HI tests as evidence of past infection or vaccination. Commercially available ELISA kits to detect antibodies against the NP or subtype-specific H5 and H7 HAs exist [5]. AGID tests are also utilized in some settings. Serological testing may feature in surveillance for prior exposure or, in certain settings, vaccination status. AIV zoonotic threats are monitored globally by the World Health Organization (WHO), the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO) [2, 4, 6].

Safety

AIVs are host-adapted, hence human cases are infrequent and transmission between human hosts is very rarely observed. To date, the H5Nx HPAIV goose Guangdong (GsGd) lineage [7] and the China-origin H7N9 LP and HPAIVs represent strains responsible for the majority of human AIV infections within the past two decades. However, other subtypes have been linked to human infection (e.g. H9N2, H5N6) [2]. The emergence of novel viruses initially requires assessment of genetic signatures associated with potential mammalian adaptation. Direct contact with infected poultry (live birds or carcasses) represents a primary risk factor for human infection with AIVs. Most human infections involving AIVs are associated with LPAIVs, with a possible explanation being that birds appear healthy and therefore no precautions are taken. Live poultry markets can provide an opportunity for such zoonotic contact. Secondary risk factors include the consumption of uncooked infected poultry products and commodities. There has been some limited suggestion of transmission from possible close contact with other human cases, particularly in hospital settings.

TREATMENT AND RESISTANCE

Treatment

Preventive measures such as strict biosecurity strategies and surveillance can be effective in halting the introduction of AIV in poultry. H5 and H7 AIVs of both pathotypes are OIE-notifiable animal disease agents [1, 7, 8], so where disease is confirmed, outbreaks must be reported to the appropriate national and international authorities, and infected flocks are usually culled. Vaccination of flocks is applied in some global locations that lack the infrastructure and logistics for rapid laboratory diagnosis and culling. Antiviral treatments against AIV may be important as a prophylactic for field staff during interventions against potential zoonotic strains but are not recommended for poultry use.

Resistance

The inherent rapid mutability of AIV genomes (genetic drift), and the possibility of co-infection leading to genetic reassortment (genetic shift), contributes to the emergence of novel viruses, which may result in the development of antiviral resistance. Immunogenic variability following antigenic drift can reduce the efficacy of vaccines matched to other AIV strains. Genetic shift and drift provide the basis for the rapid and continuous evolution observed across AIV populations. This genetic variation in turn allows AIVs to evade host adaptive immune responses, as well as posing challenges for public health antiviral and vaccine strategies.

PATHOGENIC STRATEGIES: HOST RANGE, HOST RESPONSE, TRANSMISSION, INFECTION AND VIRULENCE FACTORS

Host range

AIVs can infect a variety of domestic and wild bird species. All AIV subtypes (in all species, including mammals) are classified according to the genetic and antigenic nature of their surface glycoproteins. Despite the differentiation of H5 and H7 subtypes

into HP- and LPAIV for poultry, many historic HPAIV strains do not robustly infect waterfowl. However, the emergence of the H5Nx GsGd HPAIVs in 1996 has included continued maintenance of this lineage in waterfowl, with infections in domesticated and wild anseriforms being sub-clinical or asymptomatic. Therefore, H5Nx GsGd viruses circulate unnoticed before infecting galliformes to cause significant disease. LPAIV infection of wild birds and poultry is generally mild, although significant mortalities in poultry have been reported for some LPAIV subtypes. For example, in some areas, poultry-associated LPAIV lineages of the H9N2 subtype appear to be entirely restricted to terrestrial domestic birds [9]. Importantly, LPAIVs of the H5 and H7 subtypes can evolve into HPAIVs following mutation at the HA protein CS to allow systemic viral dissemination in an infected host as the CS alteration changes susceptibility to cleavage by host cell proteases [10]. The relationship between genetic traits associated with HP and LPAIV variants and clinical outcome in different hosts is very poorly understood [5].

Host response

Much of the host response to infection is poorly understood, although significant immune activation is often reported. Infection with AIV usually produces an antibody response, which is the primary mediator of immunity to influenza. Age-dependent factors are also thought to impact upon the outcome of infection, with young birds being more susceptible to infection and disease [11].

Transmission

Transmission occurs via exposure to infected bird excretions (including aerosols over short distances) and contact with the environment, such as contaminated food or water sources. Incursions of H5Nx GsGd HPAIVs are often associated with the movement of migratory water birds that act as reservoirs of the virus and are the key link to seasonal outbreaks [2].

Infection and virulence factors

The HA CS is a major determinant of pathogenicity and virulence in gallinaceous poultry. HA cleavage is essential for virus infectivity. The pathogenicity of H5/H7 AIVs is distinguished genotypically by CS sequencing. LPAIV strains possess a mono/di-basic CS and are cleaved extracellularly by trypsin-like proteases found in the respiratory and intestinal tract; HPAIV strains have a multi-basic CS and are cleaved by ubiquitous furin-like proteases, resulting in systemic infection and associated pathology [10]. LPAIVs and HPAIVs are differentiated phenotypically *in vivo* by intravenous inoculation of 4- to 8-week-old chickens [4].

Epidemiology

AIVs are endemic in aquatic wild bird populations, with periodic introduction into the domestic poultry production sector [12]. LPAIVs often circulate undetected, although they can be associated with mild to quite severe disease in poultry. In contrast, whilst HPAIVs often only cause mild disease in wild waterbirds, they are associated with severe outcomes in galliformes. The global reach of the GsGd HPAIVs is showcased by their emergence and subsequent intercontinental spread, originating from PR China in the mid-1990s, and that of several reassorted GsGd H5Nx subtypes, originating from East Asia from 2014 onwards; as well as the recent H5Nx outbreaks in Eurasia [1, 8].

Prevention

AIVs may survive in the environment for weeks, especially in colder climates. Prevention is achieved through strict biosecurity practices such as rapid culling of infected flocks, quarantine of newly purchased flocks, restriction on movement of poultry between farms, and disinfection of environmental sources of infection [8]. WHO biosafety guidelines stipulate use of containment level 2 (CL2) standards and CL3 laboratory practices for the handling of specimens that may contain AIV. Antigenically matched and administered vaccines can be effective in minimizing clinical signs and reducing viral shedding; however, vaccines that induce sterilizing immunity are not available for most AIV subtypes, although vaccines can reduce the infection burden and increase food security. Antigenic divergence among strains means that vaccines need to target multiple antigenically distinct subtypes. The majority of AIV vaccines used in poultry are inactivated whole-virus vaccines and are monovalent (incorporating H5 or H7 strains) or bivalent (including both H5 and H7 strains). Live influenza vaccines are not recommended due to the potential for reassortment. Other types of AIV vaccines include *in vitro*-expressed HA protein, *in vivo*-expressed HA protein via vectored systems and HA-based DNA vaccines [2].

Prevention and control are centred on 'stamping out' in countries that possess the veterinary and laboratory infrastructure to execute the necessary policies swiftly and effectively. However, derogations may occasionally include the vaccination of zoo birds and local emergency poultry vaccination, which must be accompanied by rigorous monitoring, which prompts any necessary stamping out. Inactivated AIV vaccines have been used in the USA since the 1970s, while both Asia and the Middle East have carried out large-scale vaccination plans against LPAIVs since the early 1990s [7].

Risk factors

Exposure of poultry to wild birds, particularly waterfowl of the order *Anseriformes*, is associated with high risk of viral incursion. Migratory bird movement is considered to be a significant risk factor for disease introduction, as well as movement of birds and/

or secondary spread via fomites between commercial farms (including live bird markets) [12]. Co-circulation and co-infection with diverse AIVs does occur, which can lead to genetic reassortment and subsequent emergence of a new subtype and/or genotype. Viral reassortment can occur within birds and within mammalian hosts (once stable mammalian lineages have been established) where different IAVs circulate, further extending the risk of pathogen evolution and the emergence of strains with pandemic potential. Intensive and extensive poultry farming practices, poor biosecurity and failed decontamination of premises before restocking are all factors that can increase the risk of disease spread. Moreover, LPAIVs can mutate into HPAIVs in gallinaceous birds [10], further highlighting the need for vigilant biosecurity and improved farming practices, even when dealing with seemingly healthy birds.

OPEN QUESTIONS

- (1) Can next generation vaccines offer improvement in reducing viral shedding, therefore reducing disease?
- (2) What are the molecular determinants associated with adaptation of AIVs to different poultry and wild bird hosts?
- (3) What roles do host and virus genetic factors in LPAIVs play in the emergence of HPAIV?
- (4) What roles do different gene segments play in the emergence of reassorted H5Nx HPAIVs of the continually evolving GsGd lineage?
- (5) Why do only H5 or H7 subtype viruses appear to undergo natural transition to HPAIVs?

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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