Avian Influenza

Author: Prof Celia Abolnik

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DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Clinical signs and pathology

In industrialised poultry holdings, a sharp rise followed by a progressive decline in water and food consumption can signal the presence of a systemic disease in a flock. In laying flocks, a drop in egg production is apparent. Individual birds affected by HPNAI often reveal little more than severe apathy and immobility. Oedema, visible at featherless parts of the head, cyanosis of the comb, wattles and legs, greenish diarrhoea and laboured breathing may be inconsistently present. In layers, soft-shelled eggs are seen initially, but any laying activities cease rapidly with progression of the disease. In less vulnerable species such as ducks, geese, and ratites, nervous signs including tremor, unusual postures (torticollis), and problems with co-ordination (ataxia) have been observed. During an outbreak of HPNAI in Saxonia, Germany, in 1979, geese compulsively swimming in narrow circles on a pond were among the first conspicuous signs leading to a preliminary suspicion of HPNAI.

The illness in fowls and turkeys caused by HPNAI is characterised by a sudden onset of severe signs and a mortality that can approach 100 % within 48 hours. Spread within an affected flock depends on the form of rearing: in flocks which are litter-reared and where direct contact and mixing of birds is possible, spread of the infection is faster than in caged holdings but would still require several days for complete contagion. Often, only a section of a house is affected. Many birds die without premonitory signs with the result that poisoning may be suspected in the early stage of an outbreak. It is worth noting that a particular HPNAI virus strain may provoke severe disease in one avian species but not in another: in live poultry markets in Hong Kong prior to a complete depopulation in 1997, 20 % of the fowls but only 2.5 % of ducks and geese harboured H5N1 HPAIV while all other galliforme, passerine and psittacine species tested virus-negative and only the fowls actually showed clinical disease. Ostriches infected with HPNAI do not usually display severe clinical signs, and when inoculated into chickens, ostrich HPNAI viruses do not cause severe disease at first, but rapidly become pathogenic upon passage.

LPAI has an incubation period in poultry usually of a few days (and rarely up to 21 days), depending upon the characteristics of the viral strain, the dose of inoculum, the species, and age of the bird. The clinical presentation of avian influenza in birds is variable and signs are fairly non-specific, therefore a diagnosis solely based on the clinical presentation is impossible. The signs following infection with low pathogenic AIV may be as discrete as ruffled feathers, transient reductions in egg production or weight loss.
combined with mild respiratory signs. Some LP strains such as certain Asian H9N2 lineages, that have adapted to efficient replication in poultry, may cause more prominent signs and also significant mortality.

The following diseases must be considered in the differential diagnosis of HPNAI because of their ability to cause a sudden onset of disease accompanied by high mortality or cyanosis in wattles and combs:

- velogenic Newcastle disease
- infectious laryngotracheitis (fowls)
- duck plague
- acute poisonings
- acute fowl cholera (Pasteurellosis) and other septicaemic diseases
- bacterial cellulitis of the comb and wattles

Less severe forms of HPNAI can be clinically even more confusing. Rapid laboratory diagnosis therefore, is pivotal to all further measures.

**Laboratory confirmation**

The classical method of AIV diagnosis is virus isolation in embryonated fowl eggs. Tracheal or cloacal swabs, faeces from live birds or homogenized organs of dead birds are used. The sample or pooled samples are treated with antibiotics and the clarified supernatants are then inoculated into the allantoic sac of nine to eleven-day-old embryonated specific pathogen-free (SPF) eggs, or specific antibody-negative (SAN) eggs. At least five eggs are inoculated per sample, and incubated for four to seven days at 35-37°C. Allantoic fluid is harvested from eggs containing dead or dying embryos, and then tested for hemagglutinating (HA) activity. Detection of HA activity (HA test) indicates a high probability of the presence of influenza A virus or of an avian paramyxovirus (e.g. Newcastle disease virus). The presence of influenza A virus can be confirmed in various other serological tests, including the agar gel immunodiffusion (AGID) test that demonstrates the presence of antibodies against the nucleoprotein (NP) or matrix (M) antigens, HI tests, and various commercially available ELISAs. Alternatively, the presence of influenza virus, and subtyping, can be confirmed with the use of reverse transcription polymerase chain reaction (RT-PCR) or real time reverse transcription PCR (rRT-PCR). rRT-PCR is able to detect the presence of AIV nucleic acids even if the viruses are no longer viable, and is therefore considered to be a more sensitive method than virus isolation. The matrix gene (M) is a common AIV group-specific target for rRT-PCR assays, and once presence of AI RNA is confirmed, subsequent rRT-PCR assays targeting the H5 and H7 genes are applied. Sensitive type-specific (H5/H7) conventional RT-PCR assays are used to amplify a short gene segment that spans the hemagglutinin cleavage site (HA0), and DNA sequence analysis is applied to determine the peptide cleavage signal sequence at HA0. Non-specific RNA amplification coupled with next generation sequencing technologies are increasingly being applied to determine full genomic sequences of AIVs. These techniques are providing new insights into adaptation of AIVs in the host, and have the added advantage of potentially retrieving partial or full genomic sequences directly from samples (swabs, tissues) i.e. in the absence of virus isolation, where very low
levels or viruses are present, or viruses have lost its infectivity. Nowadays, a genomic sequence is sufficient to reconstruct an infective virus using reverse genetics technology.

The intra-venous pathogenicity index (IVPI) test is used as a method of clinically assessing the virulence of AIVs. Cultivated virus is injected intravenously into each of ten six-week-old SPF chickens, and the birds are examined at 24-hour intervals for ten days. At each observation, each bird is scored (0) if normal, (1) if sick, (2) if severely sick, and (3) if dead (dead individuals are scored as (3) at each of the remaining daily observations after death). The IVPI is the mean score per bird per observation over the ten-day period. An index of 3.0 means that all birds died within 24 hours, and an index of 0.00 means that no birds showed any clinical sign during the ten-day observation period. The OIE and European Union (EU) have adopted the following definition to confirm disease for the purposes of disease control: ‘HPNAI is defined as an infection of poultry caused by an influenza A virus that has an IVPI in 6-week-old chickens >1.2 or any infection with influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of the haemagglutinin’.