Avian influenza A (H9N2) virus: from migratory flyways to cross-protection

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Influenza A viruses are known to cause infection in birds and mammals. Symptoms may vary from mild (low pathogenic avian influenza virus, LPAI) to severe (highly pathogenic avian influenza virus, HPAI), and highly lethal infections can be observed in affected flocks.

H9N2 subtype viruses, a LPAI subtype, have been recently reported in Middle East countries as responsible for widespread and serious infection problems, usually associated with an increase of economic losses.

Since avian influenza (AI) is an important zoonotic infection associated with sporadic mortality in man, eradication and control of infection in poultry flocks will reduce public health concerns.

With more than 40 years of experience, IZO contributes to prophylaxis and therapy against animal diseases using its knowledge in design, development and production of live attenuated and inactivated vaccines, both viral and bacterial.

One of the main corporate values is to supply the best solution, analysing data received from the field and guaranteeing the best standard of quality. In the last 10 years IZO has directed its attention to avian influenza viruses to provide vaccines as a control measure to prevent clinical signs in affected animals and spread in non-infected animals.

IZO produces and sells vaccines against LPAI H9 and H5 in several countries, to confer protection in poultry and to prevent spread of viruses and economic losses associated with AI outbreaks.

Analytical data are usually collected in R&D laboratories or in collaboration with universities or institutions, such as the FAO and OIE reference laboratory for avian influenza and Newcastle disease managed by Dr Capua.

A/H9N2 virus in Italy

The A/H9N2 IZOVAC strain is an LPAI virus isolated from the field in Italy. Partial sequence of HA gene, the main determinant of influenza severity, was obtained by PCR and subsequent sequencing reaction.

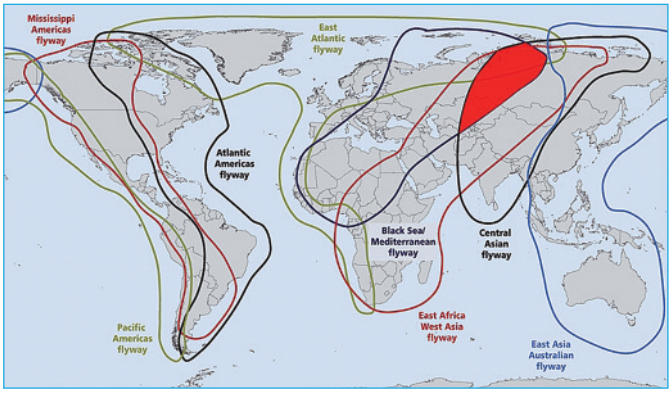


Fig. 2. Migratory flyways of the world: highlighted in red the crossroad of the Mediterranean/Black Sea Flyway, the West Asia/East Africa Flyway and the Central Asian Flyway (Wetlands International).

Sequence data were aligned and compared with all publicly available H9N2 sequences from European and Asian isolates.

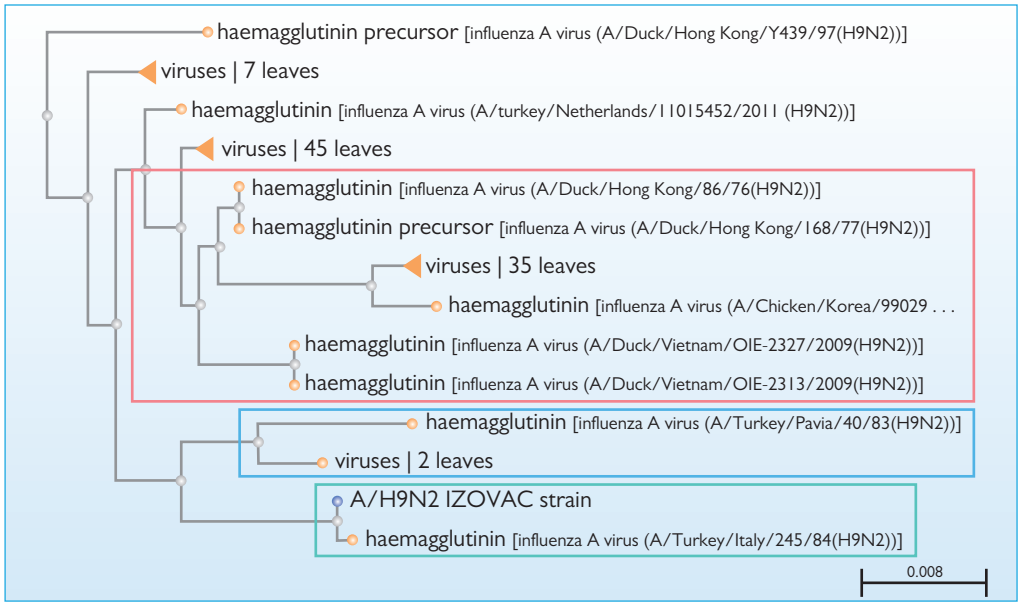
Obtained data shows a maximum identity (99%) with an H9N2 virus isolated in Italy from turkeys in 1984 (AF218089), considered as representative of an outbreak in Northern Italy in 1984-85.

The absence of multiple basic amino acid typical of HPAI subtypes in the cleavage site sequence of the precursor haemagglutinin protein is in accordance with H9N2 subtype characteristics.

The results of the phylogenetic analysis of translated sequence (Fig. 1) show a branch (green) formed from A/H9N2 IZOVAC strain and H9N2 A/turkey/Italy/245/84 isolate.

This branch is close to branches formed from Italian (blue) and Southeast Asian isolates (red).

Fig. 1. Phylogenetic analysis: A/H9N2 IZOVAC strain and H9N2 A/ turkey/Italy/245/84 isolate (green branch), Italian isolates (blue branch) and Southeast Asian isolates (red branch).



Inactivated vaccine

An inactivated vaccine against AI (IZOVAC AVIFLU H9N2) was designed using A/H9N2 IZOVAC strain virus to confer protection against H9 avian influenza virus in poultry. A haemagglutination inhibition assay (HI) was performed to measure flu-specific antibody levels in blood serum from vaccinated animals. Official OIE guidelines describe HI serological titre should be 1:32 (5 log2) as a minimum to confer protection from mortality and greater than 1:128 (7 log2) to provide reduction in virus replication and shedding.

The HI test was carried out in 10 SPF chickens vaccinated with IZOVAC AVIFLU (H9N2) and showed data are expressed as geometric mean titre (GMT). At 28 days post-vaccination, all birds showed an HI

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titre against the homologous virus greater than 5 log2, among which the 80% reached an HI titre greater than or equal 7 log2. The average HI titre observed in the vaccinated group was 7.35±0.27 Log2.

Evaluation of the haemagglutination assay reactivity against recent AI Middle-Eastern isolates show that IZOVAC AVIFLU H9N2 provide mainly clinical protection against H9N2 genogroup B, associated with an HI titer observed higher than 5 Log2.

As observed by Fusaro and colleagues (2011), genogroup B appear to be present since 2003 in several isolates of Iran, Pakistan, Jordan and Saudia Arabia, exhibiting a widespread diffusion.

Migratory flyways

Avian influenza viruses are commonly isolated in wild birds around the world. Typical flyways connects bird populations in time (migration periods) and space (breeding and non-breeding areas) and the effects of this behaviour is that virus-infected birds can transmit their pathogens bringing the viruses to new areas. As recently observed, the major breeding, feeding, and staging sites are also regions of high viral prevalence.

Cattoli and colleagues described the isolation as sub-lineage in Europe, Africa and Middle East of an avian influenza A (H5N1) Qianghai-strain virus originally isolated in China.

One possible explanation is that two of the main migratory bird flyways, the Mediterranean/Black Sea Flyway and the East Asia/East Africa Flyway, share the same non-breeding areas in Central and South Africa regions and that different sub-lineages share phylogeny with a unique virus implicated in an original outbreak. This could have led to the observed and described cross-reactivity between A/H9N2 IZOVAC strain and different H9N2 Asian isolates. ■

Phylogenetic characterisation of IB virus in Italy

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Avian infectious bronchitis is a highly contagious viral disease, able to spread very rapidly in non-protected birds, and one of the most common viral respiratory diseases in chicken.

Its viral origin (IB virus, IBV) was established in 1936 as caused by a member of the genus of coronavirus.

Typical symptoms and clinical signs can involve the upper-respiratory tract, the gastrointestinal tract, the oviduct, and the kidney.

In particular, nephropathogenic IBV strains infection can induce renal damages and this is an increasingly important feature of IB infections, especially in broilers.

Due to a high rate of mutation and recombination in the viral genome, new types of the virus continue to arise. Although in the Middle East Mass-type isolates, H120 serotype isolates, D274, IBV variant 1 and 2 strains are widely observed, recently IB QX-like strains have been isolated in different countries.

Reid S and colleagues (abstract) reported the isolation of IBV QX-like strain in Iraq and it is probably possible to speculate that they come from Europe through international trade of animals and eggs.

With more than 40 years of experience, IZO offers different solutions against several IBV strains, both inactivated and live vaccines.

Several products are developed to confer protection against IB M/41, H120 and dutch strains (D274 and D1466) infections.

Italian isolate

Originally isolated in 1986 during an outbreak of disease in the Po Valley from chickens with nephritis, IBV strain IZOVAC 28/86 was successfully attenuated after 110 passages in SPF chicken embryonated eggs to obtain no disease manifestation. Further analysis showed that this strain spread in the lungs, intestine and kidney.

To perform a genetic characterisation of the IBV strain IZOVAC 28/86, complete sequence of spike glycoprotein S1 subunit gene, due to its role in host cells adhesion and in inducing neutralising antibodies, was obtained and data were aligned and compared with all publicly available IBV sequences. Obtained data shows an homology (97%) with an IB virus isolated in Italy from chicken in 2011: isolation performed from kidney and genetic analysis showed that it belongs to the Q1 genotype. Deducted translated amino acid sequence, showed an homology between IBV strain IZOVAC 28/86 and the same Italian isolate (96%).

The results of the phylogenetic analysis of nucleotide sequence show that IB IZOVAC 28/86 strain is closely related to a branch formed from QX strains and other Chinese isolates.Further analysis was carried out starting from data obtained by Ababneh and colleagues (2012): a phylogenetic tree was built using different amino acid sequences from isolates characterising the typical symptoms of the disease (i.e. respiratory, proventriculus damages and nephritis).

Results of the phylogenetic analysis showed that IBV strain IZOVAC 28/86 belongs to a branch formed from several QX-like isolates characterised by nephropathogenic symptoms. A distinct branch of the phylogenetic tree contains IBV strains (such as H120 and M41) usually causing respiratory symptoms.

Genetic analysis

Ladman and colleagues (2006) found that the level of homology of the S1 subunit or part of it can predict cross-protection: an homology greater than 90% is related to and higher chance of cross-protection.

IBV strain IZOVAC 28/86 was originally isolated in chickens with nephritis and genetic analysis showed a phylogenetic correlation between this Italian isolate and several nephropathogenic IBV strains isolated in Asian countries.

An homology greater than the 90% permits to postulate a cross-protection induced using IBV strain IZOVAC 28/86 against nephropathogenic IBV strains ■

References are available from the author on request