Avian embryo susceptibility to Italian H7N1 avian influenza viruses belonging to different genetic lineages

Brief Report

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Received February 12, 2002; accepted April 17, 2002
Published online July 19, 2002 © Springer-Verlag 2002

Summary. In the present paper we report of the results of an immunohistochemical investigation to assess tissue tropism and viral replication in developing chicken, turkey, Muscovy duck and mallard duck embryos, of Italian H7N1 isolates belonging to different genetic lineages. LPAI isolates were chosen on the basis of the location in the phylogenetic tree: a progenitor strain, A/ty/Italy/977/V99, (exhibiting no additional glycosylation site, nAGS), strain A/ty/Italy/2379/V99 (AGS in position 123) and strain A/ty/Italy/3675/V99 (AGS in position 149) were selected. The latter two strains belonged to distinct lineages originating from the pool of progenitor strains. HPAI isolate A/ty/Italy/4580/V99 was also included in the study. All the embryos tested supported the growth of HPAI. The LPAI isolates replicated readily in the allantoic layer of the CAM of all the species tested, and did not grow in the developing chicken, turkey and Muscovy duck embryos. In contrast, they replicated to different extents in the respiratory tract of the developing mallard embryo, which also presented lower mortality rates than the other species. We conclude from these findings that the pathogenesis of LPAI infections in mallard embryos is different to that observed in other species, and should be investigated further.

Avian influenza (AI) viruses may be classified on the basis of the clinical condition they cause in susceptible birds. Low pathogenicity avian influenza (LPAI), may be caused by viruses belonging to all 15 haemagglutinin types (H1-H15) and is usually a mild disease in susceptible poultry. Highly pathogenic avian influenza (HPAI), which is caused by only certain viruses of the H5 and H7 subtypes, is, in
contrast, a devastating disease of poultry with mortality rates that approach 100% in gallinaceous birds.

Multiple basic amino acids at the cleavage site of the precursor of the haemagglutinin (HA) glycoprotein are an absolute requirement for HPAI viruses [1] while LPAI viruses only possess two basic residues at the deduced cleavage site. This difference enables HPAI viruses to be cleaved by ubiquitous proteases, among which furin appears to be the most prominent one. In contrast, the amino acid composition of the precursor of the haemagglutinin molecule of LPAI viruses enable them to be proteolytically cleaved only by trypsin-like enzymes. This diversity, results in the development of a systemic lethal disease in the case of HPAI and in a localised infection in case of LPAI, in which viral replication is restricted to the respiratory and intestinal tract epithelia in which trypsin is present [1, 15].

Similarly, growth in chicken embryos via the intra-allantoic route of AI viruses is influenced by the cleavability of the viral particles by host proteases. HPAI viruses are able to replicate in all layers of the chorioallantoic membrane (CAM) (allantoic and chorionic), to spread to the mesenchima and in the vascular endothelium, thus enabling them to gain the bloodstream, and invade the tissues of the developing embryo [16]. In contrast, the replication of viruses of low pathogenicity is restricted to the allantoic epithelium, and infectious particles are unable to gain the bloodstream and therefore their replication does not occur in the developing embryo [16, 12].

Phylogenetic studies suggest that HPAI viruses do not form separate lineages from viruses of low virulence, and this supports the current theory that they arise by mutation from viruses of low pathogenicity [2, 14], possibly after the introduction into domestic poultry from their natural reservoirs [9]. The latter, appear to be wild birds, in particular waterfowl (especially Anseriformes and Charidriiformes species), in fact, great numbers of LPAI strains have been isolated from this source [7, 8], while very few HPAI viruses have been recovered from these species.

North-eastern Italy was recently affected by a devastating epidemic of highly pathogenic avian influenza (HPAI), caused by a type A influenza virus of H7N1 subtype, which originated from the mutation of a low pathogenicity avian influenza (LPAI) virus of the same subtype [4]. Phylogenetic analyses performed on Italian LPAI and HPAI isolates [3] showed that all the 1999/2000 Italian H7N1 influenza viruses have the three glycosylation sites conserved among H7 viruses at amino acid positions 12, 28 and 231 (H7 numbering). Some have an additional glycosylation site at either amino acid position 123 or 149, both these sites lie close to the receptor binding site in the globular head of the HA. These glycosylation patterns are reflected in the phylogenetic analysis, where three branches corresponding to the acquisition of glycosylation sites appear to diverge from a common progenitor. This might suggest that there is a selective pressure on the virus from the poultry host for acquisition of glycosylation sites in the proximity of the receptor binding site, and that these mutations occurred as a result of viral adaptation to the domestic host.