

Correspondence

Pathogenicity of avian influenza H11N1 virus isolated from wild aquatic bird Eurasian Spoonbill (*Platalea leucorodia*)

Sir,

Several outbreaks of the highly pathogenic avian influenza (HPAI) H5N1 virus from poultry have been reported from India during the period spanning from 2006 to 2012¹⁻³. The National Institute of Virology (NIV), Pune, India, conducted surveys in poultry, wild and migratory birds for detection and isolation of avian influenza (AI) viruses during 2006-2010. During these surveys influenza A virus was isolated from a wild aquatic bird, Eurasian Spoonbill (*Platalea leucorodia*), Rui-Chhatrapati village, Maharashtra, India, which was identified as AI H11N1 virus⁴. The isolation of AI H11N1 virus has been recently reported in Northern pintails (*Anas acuta*) in Japan⁵. There are reports of prevalence of AI H11N1 virus from Hong Kong, the United States of America and Japan from Black duck, chicken, duck, gull, mallard and ruddy turnstone⁴.

The evidence of past influenza A H11 infection in persons who were routinely, heavily exposed to wild ducks and geese through recreational activities (duck hunting) or through their employment (bird banding) has been shown in a cross-sectional seroprevalence study⁶. In addition, serum surveillance demonstrated that Lebanese backyard poultry growers, who were frequently exposed to chickens, may have been infected with H11 AI virus. These events have indicated that H11 influenza virus may possess the ability to cross the species barrier to infect humans⁷. Therefore, in the current scenario of emerging influenza viruses, it is necessary to understand virological characteristics, pathogenesis of AI viruses to study animal-human interface. The present study was undertaken to assess pathogenicity of H11N1 virus.

The virus isolates A/Aquatic Bird/India/NIV-0717095/2007-H11N1 was used in the study (GenBank Accession Nos. CY055172 to CY055179). The virus

was inoculated in 10-day old embryonated chicken eggs (Venkateshwara Hatcheries Pvt Ltd, Pune). After incubation at 37 °C for 72 h, allantoic fluids were collected, aliquoted and stored at -80 °C for further study⁸.

Virus titration was performed by inoculating 10⁻¹ to 10⁻¹⁰ virus dilutions in 10-day old embryonated chicken eggs. Each dilution was inoculated by the allantoic route in four embryonated chicken eggs. Allantoic fluids from the inoculated eggs were harvested and tested by the haemagglutination (HA) assay. Allantoic fluids showing ≥ 2 HA titre were considered positive for virus replication. Fifty per cent egg infectious dose (EID₅₀) titres were calculated using the Reed and Muench method⁹.

The pathogenicity of virus was assessed by intravenous pathogenicity index (IVPI) assay in ten 5-week old chickens. Two chickens were kept as controls. The IVPI of AI H11N1 virus was determined as described in the Manual of Standards for Diagnostic Tests and Vaccines⁷. The Institutional Animal Ethical Committee approved the animal experiments. The chickens were housed in an animal isolator (Montair Process Technology, The Netherlands) under negative pressure. IVPI was calculated according to illness severity and the viability period following inoculation of the virus isolate.

BALB/c male mice (5-6 wk old) were used to study the pathogenicity. Mice were inoculated with 10^{4.4} EID₅₀ of H11N1 virus by intranasal (i.n.) route (50 μ l/mouse). Each mice group contained eight mice. Control mice received uninfected allantoic fluid (50 μ l/mouse). Prior to inoculation, mice were anaesthetized using CO₂ and were housed in individually ventilated cages (IVCs)¹¹. These mice were daily observed for signs of disease, mortality and weights were monitored up to the post-infection day (PID) 14.

The mice organs namely liver, spleen, heart, lung, trachea, thymus, kidney, small and large intestine, pancreas and brain were collected on PID 3 and 6. Organs were triturated to prepare 10 per cent individual suspensions in virus transport medium, diluted 1:1 v/v and were inoculated in 10-day old embryonated chicken eggs. After incubation at 37 °C for 72 h, eggs were kept at 4 °C overnight and allantoic fluids were harvested. HA assay was performed to detect the presence of virus growth. The lung suspension was further titrated to determine infections virus titre (50% egg infectious dose – EID₅₀) using embryonated chicken eggs⁹.

HA assay was performed as described in the WHO manual on animal influenza diagnosis and surveillance⁸. Blood was collected from mice on PID 14. Serum was separated and treated with receptor destroying enzyme (Denka Seiken Co., Ltd, Japan) to remove non-specific inhibitors. All serum samples were tested by haemagglutination inhibition (HI) assay using 0.5 per cent turkey RBCs to detect serum antibody levels in mice⁸.

For histopathology examination, inoculated and uninoculated control mice were euthanized on PID 3 and 6 and organs were collected in 10 per cent neutral buffered formalin. After necropsy, these tissues were immediately fixed for a minimum period of 48 h. Tissues were processed for paraffin embedding, sectioned at 4 µm, stained with hematoxylin and eosin for examination by light microscopy. Selected sections from lung, liver and spleen were also stained with periodic acid-schiff stain. Tissues examined by light microscopy included liver, spleen, heart, lung, trachea, thymus, kidney, stomach, small and large intestine, pancreas and brain.

Per cent weight loss or gain in mice was calculated by comparing average weight of eight mice in each group before virus infection with average weight of the same mice group on each PID. Per cent weight loss, mean and standard error of mean were calculated using PASW version 18 software (USA). Geometric mean titres (GMT) of antibodies were calculated for mice groups.

AI H11N1 virus grew in 10-day old embryonated chicken eggs. No mortality was observed in chick embryos. The allantoic fluid from infected eggs showed ≥ 256 HA titre.

In the IVPI assay, AI H11N1 virus did not show any signs of sickness, respiratory illness or mortality

in the inoculated chickens during 10 days observation period. The IVP index was 0.0/3.0. Thus, this was low pathogenic avian influenza (LPAI) virus in chickens.

No mortality was observed in infected mice. Mice showed weight loss up to PID 7 and remained underweight till PID 14. None of the negative control mice exhibited weight loss. The virus was isolated from lungs and trachea of infected mice. All other organs were negative for virus isolation. The lungs of inoculated mice showed 2.19 and 1.25 EID₅₀/100 µl infectious virus titre on PID 3 and PID 6, respectively. Infected mice were positive for presence of antibodies by HI assay on PID 14, showed antibody titre ranging from 10 to 40.

AI H11N1 virus showed pathological changes only in the airways and lungs; no specific abnormalities were identified in other organs. In the respiratory system, pathological lesions caused by H11N1 virus were less severe. The histopathological findings observed in the lung tissues of infected mice were focal non-coalescent small areas of consolidation, mild intra-alveolar, intrabronchiolar, intraseptal, and interstitial mononuclear cell (MNC) inflammatory infiltrate. Peribronchiolar and perivascular cuff of mild MNC infiltrate was also evident. Bronchiolar epithelial cells appeared taller with apical capping of secretions. The alveoli adjacent to the bronchioles showed mild congested blood vessels with accumulation of occasional lymphocyte and macrophage in the alveolar lumina. Alveolar septa appeared broadened and thickened due to mild MNC infiltration and dilated and congested blood vessels. Features were not up to the severity to satisfy the definition of diffused alveolar damage. Trachea showed mild MNC infiltrate in submucosa with intact mucosa and scant inflammatory exudates in the lumen. Lungs and trachea from uninoculated mice showed normal histology (Fig.).

There are no data available on AI H11N1 virus pathogenicity in chickens and mice. The present study showed that the virus infected chickens did not show any sickness and mortality, thus the virus was of low-pathogenicity. A mouse is often used as a model to study influenza viruses; however, mice differ in their susceptibilities to human influenza virus strains¹². The H11N1 virus caused mild infection with weight loss and mice remained underweight as compared to uninfected mice. No mortality was seen. The mice organs other than lungs were negative for virus isolation, confirming that this virus did not cause

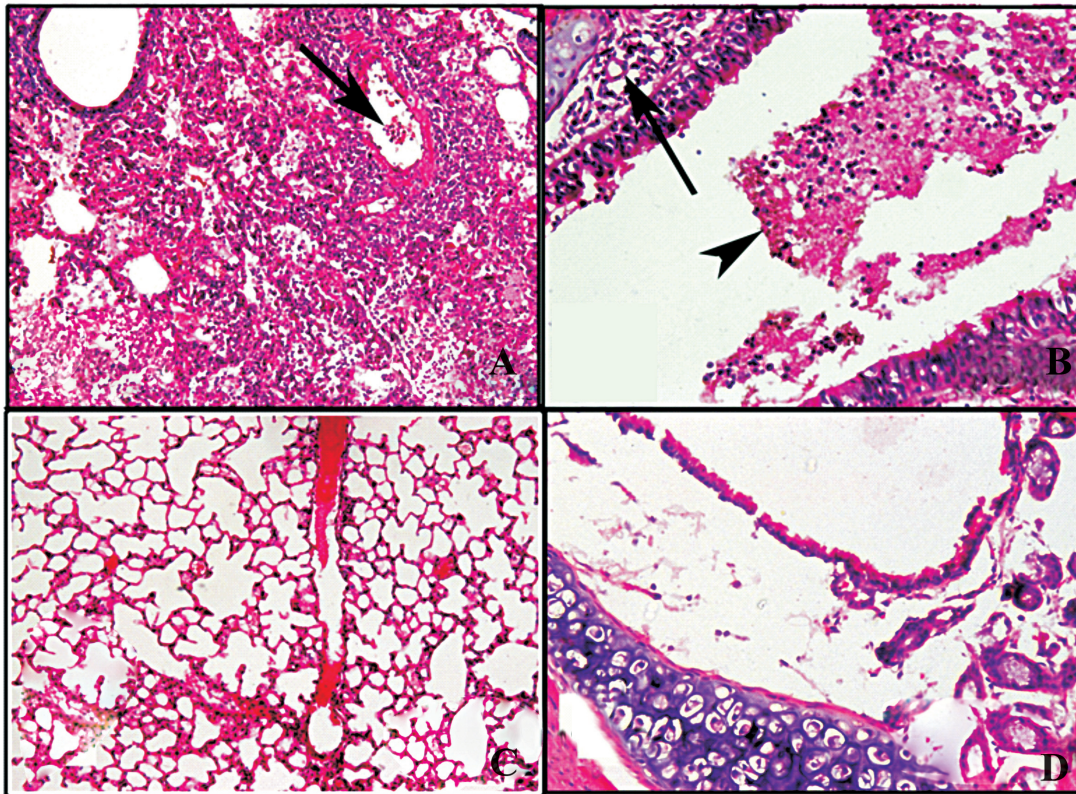


Fig. Histopathologic features in lungs and trachea of mice experimentally infected with AI H11N1 virus. **A.** Lung, H11N1-infected mice: Focal non-coalescent small areas of consolidation, mild intra-alveolar, intrabronchiolar, intraseptal, and interstitial mononuclear cell (MNC) inflammatory infiltrate. Peribronchiolar and perivascular cuff of mild MNC infiltrate was also evident. **B.** Trachea, H11N1-infected mice: Trachea showed mild MNC infiltrate in submucosa with intact mucosa and scant inflammatory exudates in the lumen. **C.** Lung, un-infected mouse: Normal histology. **D.** Trachea, un-infected mouse: Normal histology. All sections are H-E stained, original magnifications, A-D: 200X.

systemic infection. Histologically, the virus under study showed predominant changes in the airways and lungs.

Tests for binding properties of various types of influenza viruses to sialic acid, sialoligosaccharides or gangliosides, have indicated that the binding pattern to different gangliosides by H11N9 viruses resembled most human viruses rather than avian isolates¹³. In receptor specificity assays, the H11N1 virus under study showed ability to bind both avian and mammalian sialic acid (SA α 2, 3-Gal and SA α 2, 6-Gal) receptors¹⁴. The seroprevalence of H11 virus among Waterfowl hunters, wildlife professionals and chicken growers highlight probable potential of H11 viruses to cross species barrier⁷. Therefore, the present study underlines the need of continuous AI surveillance in wild and migratory birds and studies on animal-human interface.

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