SHORT COMMUNICATION

Veterinary Research Forum. 2022; 13 (3) 439 – 441
doi: 10.30466/vrf.2020.119136.2818
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Apoptosis in field and experimental cases of avian influenza H_9N_2 infection in broiler chickens in Iran

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Article Info

Article history:

Received: 25 December 2019
Accepted: 06 July 2020
Available online: 15 September 2022

Keywords:

Apoptosis
H_9N_2 avian influenza
Microscopy
Trachea

Abstract

Avian influenza virus subtype H_9N_2 is widely circulating around the globe affecting many species of animal including mammals and birds as well as human beings. The virus has pandemic potential due to segmented nature of the viral genome. Ultra-structural features of apoptosis in field and experimental infection of H_9N_2 avian influenza virus were studied. Freshly dead birds from affected broiler farms and experimentally infected broiler chickens with H_9N_2 subtypes were subjected to routine necropsy. Post-mortem findings in different organs were recorded. Appropriate specimens from the trachea were taken for electron microscopy studies. In electron microscopy study, frequent apoptotic bodies were observed in the epithelial cells of the trachea. Increase of antibody titer to H_9N_2 virus following challenge with the virus in experimental group indicates that the infectious cycle has been initiated in the affected birds.

Introduction

The H_9N_2 avian influenza infection is one of the most worrisome infections with pandemic potential circulating in many parts of the world especially in Eurasia region.[1] It can infect many species of mammals and birds.[2] Poultry workers become seroconverted in affected poultry farms[3] meaning that human beings have receptors compatible with the virus which can initiate infectious cycle in an affected person. Considering the re-assortment ability of the virus, this could be very dangerous phenomenon from the public health point of view. Different aspects of the disease have been studied during last two decades;[2,4-6] however, there is no report regarding the ultra-structural features of H_9N_2 avian influenza infection.

Apoptosis is one of the basic mechanisms for cell death in many pathological pathways. It has been shown that H_9N_2 and H_5N_1 viruses induce apoptosis in the oviduct of laying hens and alveolar epithelia of the human lung, respectively.[4-7]Also, the H_9N_2 low pathogenic avian influenza virus which is the most widespread avian influenza (AI) subtype worldwide, can induce apoptosis in chicken macrophages through the Fas/(FasL)-mediated extrinsic pathway.[5-8]

In this study, electron microscopy technique was used to show extensive apoptotic bodies in tracheal epithelial cells in field cases and experimentally infected broiler chickens with H_9N_2 avian influenza virus (AIV) subtype.

Materials and Methods

Field cases; freshly dead birds from 30 affected flocks were subjected to routine necropsy. Flocks subjected to this study had Newcastle disease vaccination program; all body systems were examined, lesions were recorded, appropriate specimens from selected flocks were taken for electron microscopic studies and blood samples were collected from live cases for serological analysis. All experiments involved animal handling and manipulation were conducted based on guidelines of animal ethics committee code of practice (83-VE-1509-102) of Shiraz University Research Committee, Shiraz, Iran.

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Experimental design. Two hundred and forty 1-day-old broiler chicks were divided randomly into two challenged and unchallenged groups. Each group was subdivided into two equal subgroups (A and B) being housed in pens separated by metal fence. Chickens were monitored daily for general conditions such as growth rate, food and water consumptions and presence of clinical signs. In groups 1 and 2, one-half of the birds (subgroups A) were vaccinated at the 10th day of age, subcutaneously at the back of neck, with 0.50 mL chicken embryo-propagated and beta-propiolactone inactivated oil-emulsion vaccine containing H_9N_2 AIV (Razi Institute, Karaj, Iran). The H_9N_2 virus was prepared using the method described by Nili and Asasi and at 21 days of age birds were challenged with the virus.[6]

Serology. Hemagglutination inhibition (HI) test using antigen from H_9N_2 AIV (courtesy of Razi Institute) was used to detect anti-influenza antibodies in field (30 flocks) and experimental cases. In the experimental study, sera were obtained from all chickens in groups 1 and 2 at 41 days of age and examined for HI antibodies against H_9 AIV.

Transmission electron microscope (TEM). One cubic mm of tissues from the tracheal epithelium of freshly sacrificed birds, using cervical dislocation, were fixed in 3.50% glutaraldehyde, post-fixed in 1.00% osmium tetroxide, dehydrated in ascending concentrations of ethanol and embedded in medium Spurr's resin. Survey sections (1.00 μm) were stained with toluidine blue and examined for trimming of the blocks to areas of interest. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined through Philips CM10 TEM (Philips, Eindhoven, The Netherlands).

Results

Field study. The primary clinical signs were anorexia and reduced water intake, which followed by depression, coughing, sneezing and trouble in breathing. Severe damage of body weight gain was also reported. In cases of co-infection with other respiratory infectious organisms, the morbidity and mortality were much more severe. Enlargement of the sinuses and contamination of nearby nostril due to discharge from the eyes were common in field cases. The most prominent clinical signs were dyspnea and affected birds gasped for air with the mouth open, head raised and neck extended. A squeaking sound could be heard especially during the night.

The maximum severely affected birds died suddenly from asphyxiation as a result of necrotic debris occluding the airway at the tracheal bifurcation. Mortality in affected flocks was between 20.00 and 65.00% during 5 to7 days following the presence of the first clinical signs. During the acute phase of infection, injuries were identified primarily in the respiratory tract. The nasal conchae and upper respiratory tissues were severely congested. In some cases, the lung was also showed different degrees of congestion. Cast formation in the tracheal bifurcation was one of the most prominent post-mortem findings. Thorough or fractional blockage of the tracheal branching resulted in asphyxiation in some severely affected birds. Fibrinous-purulent exudates was present on the tracheal mucosa, larynx and in some cases in or pharynx.

Transmission electron microscopy study. Ultra-structural study showed frequent apoptotic bodies in the tracheal epithelium of field cases and birds from experimental groups (Fig. 1). Features observed in experimental cases mimicked the same pathological patterns, as seen in a natural outbreak. Virus replication through a budding process on the tracheal epithelium was similar to the field cases. Shrinkage of cellular organelles and dilatation of the others were observed in apoptotic bodies inside cell cytoplasm (Fig.1).

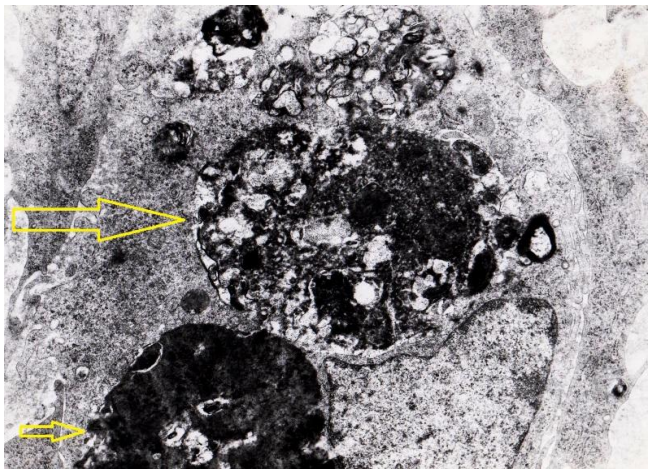

Fig. 1. Frequent apoptotic bodies (short and long arrows) observed in one the tracheal epithelial cell of field cases and birds from experimental groups. Shrinkage of cellular organelles (short arrow) and dilatation of the others (long arrow) were observed in apoptotic bodies inside cell cytoplasm (Uranyl acetate and lead citrate staining, 12000×).

Serological study. Results of serological study are presented in Table 1. No antibody response to Newcastle disease shows that there has not been exposure to the virus in different groups.

Table 1. Hemagglutination inhibition antibody responses (mean ± SD log_2 of the antibody titer) of vaccinated challenged and unchallenged groups.

Viruses	Group 1 (challenged)		Group 2 (unchallenged)	
	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated
Avian Influenza H_9N_2 virus	6.50 ± 1.40	6.38 ± 1.70	5.38 ± 1.70	0.00
Newcastle disease virus	0.00	0.00	0.00	000

Discussion

Transmission electron microscopy is a powerful tool elaborating the ultra-structural features of cellular organelles in health status and disease condition. High resolution at high magnification shows details of cellular organelles and morphological changes occurring inside the cell. In this study, ultra-structural features of apoptosis in H_9N_2 AIV infection were examined in field and experimental cases of disease. Observation of several apoptotic bodies with different morphological features in one epithelial cell indicates the extensiveness of the process during the course of disease. Increase of antibody titer to H_9N_2 virus following challenge with the virus in experimental group, especially in unvaccinated challenged group, indicates that the infectious cycle has been initiated in the affected birds.

It has been shown that different body systems such as respiratory, digestive and reproductive tracts in chickens affected by H_9N_2 AIV show symptoms of mild bleeding.[10-11] However, there is no report regarding the ultra-structures of lesions. Excessive cellular apoptosis and tissue damages have been suggested to occur in the reproductive tract of laying hens following infection with H_9N_2 AIV.[4] Apoptosis in human alveolar epithelial cells has been reported to occur following *in vitro* infection of the cells.[12]

In conclusion, observation of several apoptotic bodies with different morphological features in one epithelial cell indicates the extensiveness of the process during the course of disease.

Acknowledgments

Financially, the study has been supported by the University of Shiraz, Shiraz, Iran.

Conflict of interest

The authors declare that they do not have conflict of interest.

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