http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (18): 900-903, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.900.903

Fowl Adenovirus (FAdV) in India: Evidence for Emerging Role as Primary Respiratory Pathogen in Chickens

[1]V. Gowthaman, [1]S.D. Singh, [1]K. Dhama, [1]R. Barathidasan, [1]M. Asok Kumar, [1]P.A. Desingu, [2]N.K. Mahajan and [3]M.A. Ramakrishnan
[1]Avian Diseases Section, Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India
[2]Department of Veterinary Public Health and Epidemiology, LLRUVAS, Hisar, India
[3]Indian Veterinary Research Institute, Muktheswar, India

Abstract: Adenoviruses have been isolated from both clinically healthy and diseased birds worldwide. The pathogenic role of most of the FAdVs is still questionable. They can quickly take on the role of opportunistic pathogens when additional factors, particularly concurrent infections, adversely affect the health of the avian host. Immnosuppressing agents especially chicken infectious anemia and infectious bursal disease viruses are known to enhance the pathogenicity of FAdVs upon coinfection. The aim of the present study was to screen for the involvement of FAdV in poultry flocks affected with respiratory disease complex by RT-PCR. The samples were also screened by RT-PCR/PCR for other respiratory pathogens. Thirty two commercial poultry flocks with the history of respiratory disease complex from various parts of India. FAdV nucleic acid could be detected in tissue samples of 13 out of 34 farms investigated. Out of 13 FAdV positive farms, FAdV and CIAV were alone detected in 4/13 (31%) whereas, in other farms more than two respiratory pathogens were detected together. CIAV was detected in all the farms (34/34) investigated. Eosinophilic intranuclear inclusion bodies were noticed in FAdV infected laryngeal and tracheal epithelium under light microscopy. The findings of the study assert that FAdV can play the role of primary respiratory pathogen in immunocompromised birds and also in the presence of other respiratory pathogens.

Key words: Adenovirus, chicken, PCR, histopathology

INTRODUCTION

Adenoviruses are ubiquitous infectious agents in commercial poultry flocks and wild birds worldwide. Adenoviruses have been isolated from both clinically healthy and diseased birds (Adair and Fitzgerald, 2008). The pathogenic role of most of the FAdVs is still questionable (Hess, 2000). FAdVs can become opportunistic pathogens especially in case of concurrent infections, severely affecting the health of affected birds (Adair and Fitzgerald, 2008). Some strains of FAdV can be pathogenic in immunocompromized hosts as in Chicken Infectious Anemia (CIA) or Infectious Bursal Disease (IBD) affected birds (Adair and Fitzgerald, 2008). Clinical and gross pathological findings in mixed infections involving infectious bronchitis (IB), Mycoplasma and FAdVs can be similar to infectious laryngotracheitis (Schmidt *et al*., 1970). Lesions similar to that occur in experimental adenoviral infections were also found in natural outbreaks suggesting their significance in inducing respiratory disease (Dawson *et al*., 1980; Adair and Fitzgerald, 2008). FAdV type 1, 4, 5, 6 and 12 have been isolated in association with respiratory diseases of poultry (Meulemans *et al*., 2001). The aim of the present study was to screen for the involvement of FAdV in poultry flocks affected with respiratory disease complex by RT-PCR. The samples were also screened by RT-PCR/PCR for other respiratory pathogens viz. Newcastle disease virus, infectious bronchitis Virus (IBV), Infectious Laryngotracheitis Virus (ILTV), influenza A virus, *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS) and CIAV.

MATERIALS AND METHODS

Study area and chickens: Thirty two commercial poultry flocks with the history of respiratory disease complex from various parts of India viz. Uttar Pradesh, Haryana, Rajasthan and Tamil Nadu, were included in this study. Selected flocks consisted of multi aged layers and broilers. The layers and broilers were maintained under cage system and deep litter system of rearing. All the flocks were vaccinated against several respiratory pathogens.

Corresponding Author: V. Gowthaman, Avian Diseases Section, Division of Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India

Collection of clinical samples: Necropsy was carried out on fresh carcasses and euthanized ailing birds with the history and symptoms of respiratory diseases. The lesions were photographed and gross findings were recorded. Tissue samples such as trachea, lungs, air sacs and spleen were collected aseptically for isolation studies and direct tissue PCR. Small pieces of tissue samples from the above visceral organs were also preserved in 10% buffered formal saline for histopathology.

PCR amplification: Viral DNA from tissue homogenate was extracted using Wizard® Genomic DNA Purification Kit (Promega, USA) as per the manufacturer's instructions. PCR was carried out for Hexon gene of FAdV using primers FadV-Hexon A-CAARTTCAGR CAGACGGT and FadV-Hexon B-TAGTGATGM CGSGACATCAT (Meulemans *et al*., 2001) with the following cyclic conditions viz. initial denaturation at 95°C for 15 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and final extension at 72°C for 10 min. The PCR amplicons were visualized in 1.5% agarose gel stained with ethidium bromide (5 μg mL^{-1}).

RESULTS

Clinical signs: The general clinical signs consisted of depression, somnolascence, a reduction in normal vocalizations, huddling, drooped wings, ruffled feathers, reluctance to stand or move, facial edema, cyanotic combs and wattles, reduced feed intake and water consumption, uneven growths, runting and stunting syndrome, hock sitting posture, watery white diarrhea and severe respiratory distress. The degree of respiratory signs ranged from mild respiratory distress to asphyxia depending on the severity of the disease. These included: labored breathing, increased rales and wheezing, open mouth breathing and mucus/tenacious nasal discharge. Mortality rate in RDC affected flocks ranged between <1 and 60 %. In FAdV and CIAV coinfected flocks mortality ranged between <1 and 10%.

Gross lesions and histopathological findings: The lesions were largely confined to respiratory system and abdominal cavity that include cachexia, haemorrhagic tracheitis, caseous plaques occluding tracheal lumen (Fig. 1), severe diffuse pulmonary edema, congestion and consolidation, fibrino-necrotic pneumonia and pleurisy, airsacculitis, adhesive pericarditis and fibrinous perihepatitis. The histopathological alterations consisted of mild to severe tracheitis causing necrosis and denudation of lining epithelium with abundant

Fig. 1: Lungs and trachea of FAdV (and CIAV) infected bird. Note edematous and congested lungs and caseous plaques in the tracheal lumen

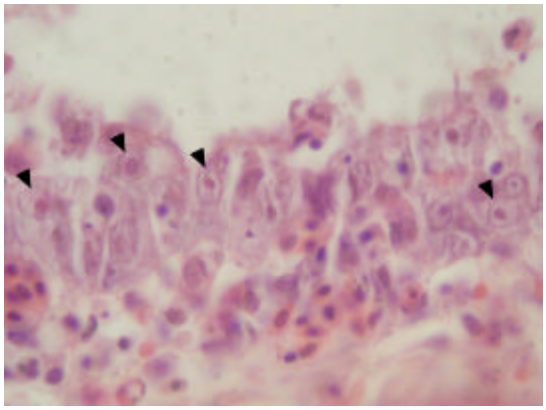

Fig. 2: Tracheal epithelium of FAdV (and CIAV) infected bird. Note large, single and regular eosinophilic I/N inclusion bodies (black arrow heads) surrounded by a clear halo in the nuclei of several swollen tracheal epithelial cells (H and Ex1000)

mononuclear cell infiltrate, fibrinous necrotizing broncho pneumonia, fibrino haemorrhagic airsacculitis and lymphoid depletion in spleen. Eosinophilic intra nuclear (I/N) inclusions typical of adenovirus infections were noticed in affected laryngeal and tracheal epithelium (Fig. 2) of birds coinfected with FAdV and CIAV alone and in cases where other respiratory pathogens are also detected.

Screening for FAdV and other respiratory pathogens: A total of 122 tissue samples (from 34 farms) were tested for the presence of FAdV and others. FAdV nucleic acid (amplicon size-897 bp) (Fig. 3) could be detected in tissue samples of 13 out of 34 farms. Out of 13 FAdV positive farms, FAdV and CIAV were alone detected in 4/13 (31%) whereas, in other farms more than two respiratory

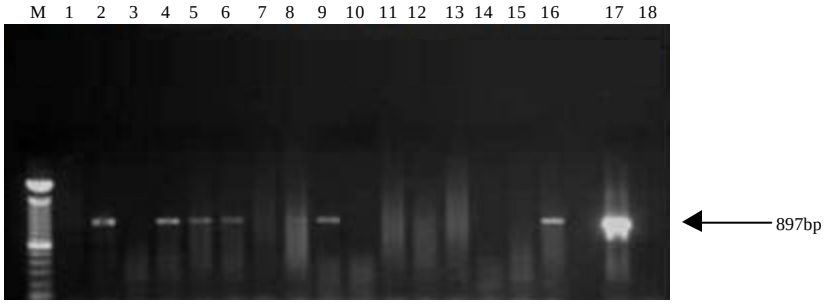

Fig. 3: FadV specific amplicons visualized by agarose gel electrophoresis, Lane M: Molecular weight marker, Lanes 1-16: Field samples, Lane 17: Positive control, Lane 18: Negative control, Lanes 2, 4, 5, 6, 8, 9 and 16 show the FAdV-specific 897 bp product while lanes 1, 3, 7, 11, 12, 13, 14 and 15 shows no amplified product and were considered negative

pathogens were detected together (data not shown). CIAV was detected in all the farms (34/34) investigated. Out of 13 FAdV-positive farm samples five were from growers and seven were from broilers.

DISCUSSION

In this study, FAdV was detected from 13 out of 34 obtained from flocks with the history of RDC. Findings of present study reconfirms the earlier reports of avian adenoviruses association with respiratory disease (McDougall and Peters, 1974; Kawamura *et al*., 1963; Lim *et al*., 1973; Meulemans *et al*., 2001). Besides, 31% (4/13) of the farms had only FAdV and CIAV suggesting that FAdV played the role of opportunistic pathogen causing respiratory disease in immunocompromised birds. This is in agreement with the earlier reports where CIAV infection helping FAdV in causing respiratory or hepatic disease were noted (Von Bulow *et al*., 1986; Toro *et al*., 2000; Adair and Fitzgerald, 2008). Presence of typical adenovirus I/N inclusions in laryngeal and tracheal epithelium in birds coinfected only with CIAV implies that FAdV played a major role in causing severe respiratory disease. FAdV and CIAV coinfected birds showed severe catarrhal to mucopurulent tracheitis. Also, the active role of FadV in causing RDC in flocks infected with more than two respiratory pathogens could not be ruled out. Because the role of subgroup I adenoviruses as primary pathogens is not clearly established, factors determining pathogenicity are not clear. Different serotypes and even strains of the same serotype, can vary in their ability to produce illness and death (Adair and Fitzgerald, 2008). Coinfection of IBDV also known to enhance the pathogenicity of some aviadenoviruses (Rosenberger *et al*., 1975; Fadly *et al*., 1980).

The findings of the study assert that FAdV can play the role of primary pathogen in immunocompromised birds and also in the presence of other respiratory pathogens. Currently, vaccination or regular monitoring of FAdV (causing respiratory disease) and Chicken Infectious Anemia Virus (CIAV) does not exist in India. It is clear that to prevent or control FAdV-induced respiratory disease, proper vaccination for other respiratory pathogens and control of immunosuppressing pathogens (mainly CIAV and IBDV) is essential. Further research to explore the genetic diversity of FAdVs associated with RDC will shed more light on the FAdV type and strains involved in causing RDC and will immensely help in vaccination programmes against FAdV if attempted.

REFERENCES

Adair, B.M. and S.D. Fitzgerald, 2008. Group I Adenovirus Infections. In: Disease of Poultry, Saif, Y.M., A.M. Fadly, J.R. Glisson, L.R. McDougald, L.K. Nolan and D. E. Swayne (Eds.). 12th Edn. Iowa State University Press, Ames, IA., USA., pp: 252-266.

Dawson, G.J., L.N. Orsi, V.J. Yates, P.W. Chang and A.D. Pronovost, 1980. An enzyme-linked immunosorbent assay for detection of antibodies to avian adenoviruses and avian adenovirus-associated virus in chickens. Avian Dis., 24: 393-402.

Fadly, A.M., B.J. Reigle, K. Nazerian and E.A. Stephens, 1980. Some observations on an adenovirus isolated from specific pathogen free chickens. Poult. Sci., 59: 21-27.

Hess, M., 2000. Detection and differentiation of avian adenoviruses: A review. Avian Pathol., 29: 195-206.

Kawamura, H., T. Sato, H. Tsubahara and S. Isogai, 1963. Isolation of CELO virus from chicken trachea. Natl. Inst. Anim. Health Q., 3: 1-10.

Lim, A.C., A. Mustaffa-Babjee, B.S. Bains and P.B. Spradbrow, 1973. An avian adenovirus associated with respiratory disease. Avian Dis., 17: 690-696.

McDougall, J.S. and R.W. Peters, 1974. Avian adenoviruses: A study of 8 field isolates. Res. Vet. Sci., 16: 12-18.

Meulemans, G., M. Boschmans, T.P. van den Berg and M. Decaesstecker, 2001. Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. Avian Pathol., 30: 655-660.

Rosenberger, J.K., S. Klopp, R.J. Eckroade and W.C. Krauss, 1975. The roles of the infectious bursal agent and several avian adenoviruses in the hemorrhagic-aplastic-anemia syndrome and gangrenous dermatitis. Avian Dis., 19: 717-729.

Schmidt, U., H. Hantschel, P. Schulze and H. Linsert, 1970. Untersuchungen uber eine mischinfektion von aviarem adenovirus und dem virus der infektiosen bronchitis [Investigations of a mixed infection of adenovirus aviarem and the virus of infectious bronchitis]. Arch. Exp. Vet. Med., 24: 587-607.

Toro, H., C. Gonzalez, L. Creda, M. Hess, E. Reyes and C. Geisse, 2000. Chicken anemia virus and fowl adenoviruses: Association to induce the inclusion bodyhepatitislhydropericardium syndrome. Avian Dis., 44: 51-58.

Von Bulow, V., R. Rudolph and B. Fuchs, 1986. Folgen der doppelinfektion von kuken mit adenovirus oder reovirus und dem erreger der aviaren infektiosen anamie (CAA) [Follow the dual infection of chickens with adenovirus or reovirus and the causative agent of avian infectious anemia (CAA)]. J. Vet. Med. Ser. B, 33: 717-726.