

Full Length Research Paper

Prevalence and clinical pathology caused by infectious bronchitis virus in poultry birds at Sindh, Pakistan

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Poultry is a major meat and eggs producing sector that generates high income throughout the world. Despite worldwide growth of poultry, it is influenced by various infectious pathogens especially infectious bronchitis virus (IBV). As a result of heavy economic losses through high morbidity, mortality, deficient performance, low weight gain, abnormal colour and misshapen eggs, this study is designed on identification and quantification of infectious bronchitis virus. A total 4000 tissue specimen (trachea, kidney and swabs) were collected from flocks with respiratory illness, from various areas of Sindh. The specimens were cultured in 10 days old specific pathogen free (SPF) embryonated chicken eggs and amnio-allantoic fluid (AAF) was harvested. Hemagglutination assay (HA) with trypsin showed slightly higher prevalence of IBV in layers ($\geq 61.2\%$) than in broilers ($\geq 52\%$). ELISA revealed that 84.4% samples were positive, while 14.6% samples were negative for IBV. It is concluded that IBV is highly prevalent in various parts of Sindh province of Pakistan, therefore time to time vaccination is required to prevent heavy economic losses.

Key words: Enzyme linked immunosorbent assay (ELISA), hemagglutination assay (HA), infectious bronchitis virus (IBV), infectious bronchitis virus, poultry, prevalence.

INTRODUCTION

Poultry is a rapid growing sector throughout the world. It has been found that market share poultry meat is about 2 to 2.5 in 1971 that has been increase to 25% in 2010 (GOP, 2013). It is economical to stockholders due to large farming size, quick output, genetic improvement and improvement in feed stuff (Martinez, 2002; Morrison et al., 2004). Despite worldwide growth of poultry, it is influenced by various infectious pathogens including

infectious bronchitis virus (IBV) (Sandhu et al., 2009).

Infectious bronchitis virus (IBV) is an acute and highly contagious virus (Bourogâa et al., 2009, Abdel-Moneim et al., 2012) causing respiratory disease in poultry. It is associated with sneezing, gasping, tracheal rales, coughing, puffy swollen eyes and inflamed sinus with poor weight gain (Sediek, 2010). The virus severely damage epithelium of trachea which leads to tracheal

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Table 1. Sample collection for IBV isolation.

Location	Type of flock	Age of flock	Number of sample	Collection date
Karachi			400	10-15/11/2015
Thatta			400	15-25/11/2015
Mirpur khas			400	25-30/11/2015
Larkana	Broiler	5-7 weeks	400	01-10/12/2015
Sukkur			400	10-20/12/2015
Karachi			400	20-30/12/2015
Thatta			400	01-05/12/2015
Mirpur khas			400	05/10/01/2016
Larkana	Layers	35-67 weeks	400	10-20/01/2016
Sukkur			400	20-30/01/2016

haemorrhages, congested lungs, air sacs and seromucus exudates on lungs as well as on air sacs (Sediek, 2005). Drop in egg production, poor quality eggs, misshapen, broken and weak shelled eggs (Seidek, 2010) followed by reduction in egg production up to 50% (Biswas, 2004) and loss shell colour (Cook and Huggins, 1986). The infection is responsible for causing morphological and histopathological changes in oviduct which influence laying of eggs (Jane et al., 2012). Prevalence of infectious bronchitis virus has been increased that lead heavy morbidity and mortality. The prevalence of infectious virus in commercial poultry is about 82.43% (Hadipour et al., 2011).

Outbreaks of IBV have been increased in last few decades in countries such as Tunisia, Egypt and Asia that caused heavy economical losses (Yu et al., 2001). Keeping in view the high morbidity and mortality due to recent outbreaks of Taiwan Group I CK/CH/LDL/971 strains of IBV (Chen et al., 2010; Jinling et al., 2012), this study is designed to investigate the prevalence and clinical pathologies caused by infectious bronchitis virus (IBV) in Pakistan.

MATERIALS AND METHODS

Sample collection and transportation

A total of 4000 tissue specimens (trachea, kidney and tracheal swabs) were collected from flocks with respiratory illness from various districts of Sindh (Karachi, Thatta, Mirpur khas, Larkana and Sukkur), from November 2015 to January 2016. In order to investigate prevalence and quantification of IBV samples were taken from 200 farms (broiler and layers with 1:1 shown in Table 1). Samples were transferred in test tubes containing sterilized phosphate-buffered saline (PBS) supplemented with penicillin (10,000 IU per ml), streptomycin (10,000 ug ml⁻¹) and nystatin sulphate (1000 IU ml⁻¹) as described by Mahmood et al. (2004). The samples were shifted to research and development laboratory and preserved at -80°C.

Virus isolation

One gram of sample was homogenised in 700 ul of PBS, after

adding 35 ul of antibiotics. The supernatant was injected to 10 day-old embryonated chicken eggs, amnio-allantoic fluid was harvested 72 h post inoculation and it was subjected to rapid HA test as described by Doherty (1967). A volume of 125 ul of samples with negative rapid HA was treated with 25 ul of trypsin then micro HA was performed (Mahmood et al., 2004).

ELISA

An antigen ELISA kit was used to detect IBV that contained antibody coated microtiter plate wells. A volume of 40 ul of tissue homogenate mixed with 10 ul of sample diluent, poured in wells and incubated for 30 min. After the addition of horseradish peroxidase (HRP) conjugate antigen and antibody complex was generated. Chromogen dye followed by stop solution was added the blue color changed to yellow color after incubation in dark room, optical density (OD) value was calculated at wavelength of 450 nm by using automatic ELISA reader after that (S/P) ratio (absorbency of sample / absorbency of positive control) was determined as described by Wang et al. (2002) and Chen et al. (2003). The samples with S/P ratio ≥ 0.2 were considered positive for IBV.

RESULTS

Results explored that Infectious bronchitis virus (IBV) is characterised by general respiratory signs such as severe conjunctivitis, lacrimation, gasping, sneezing, watery eyes, tracheal rales and coughing. Post-mortem lesions include damaged epithelium of trachea, tracheal haemorrhages, sero-mucus exudate in trachea and congested lungs with prominent infiltration of sero-mucus exudates (Figure 1a to d).

Additionally, it has been investigated that kidneys were severely congested, inflamed, pale and distended with prominent urates leading peri hepatitis and aracialities. Moreover, proximal tubules and convoluted tubules were inflamed (Figure 2).

Hemagglutination assay (HA) with trypsin in broiler flocks have found that IBV is highly prevalent at Thatta (61%), lowest at Sukkur (41%), Karachi (55%) and Larkana (45%) (Figure 3a).

Similarly, HA titers with trypsin in layer flocks have

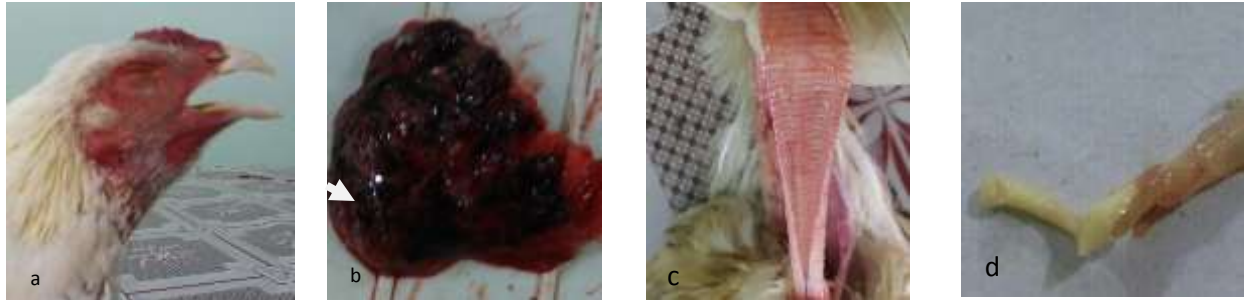


Figure 1. IBV infected birds. (a) Tracheal rales with respiratory distress. (b) Congested lungs with severe infiltration of sero-mucus exudate

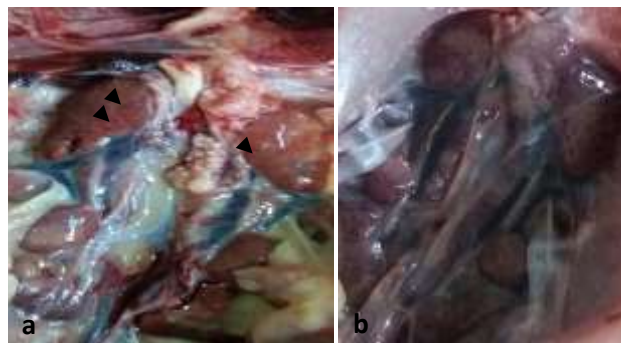


Figure 2. Kidney of IBV infected laying hen. (a) Deposition of urated in kidney and tubules. (b) IBV infected kidney bulged from renal cavity.

shown that IBV is higher in Thatta (69%) followed by Karachi, Mirpurkhas, Sukkur and Larkana (67, 62, 57 and 51%) as shown in Figure 3b.

Results have found that IBV reduced egg production; poor quality eggs, weak shelled eggs, misshaped eggs and cracked egg (Figure 4a to d). Similarly, it damaged the ovary as a result egg yolk is evenly found in abdominal cavity (Figure 4b).

Enzyme linked immunosorbent assay (ELISA) of 90 randomly selected samples showed that IBV were strongly positive 4/90 (4.44%), positive 46/90 (11.11%), weak positive 33/90 (36.66%) and negative 7/90 (7.77%) as shown in Figure 5.

DISCUSSION

Despite expeditious growth of poultry in Pakistan, this is the first study on overall prevalence of IBV throughout Sindh. The current study had determined that IBV is a major respiratory pathogen that causes catastrophic morbidity; mortality leads to heavy economic loss. The study investigated that IBV infected birds characterized by signs, that is, severe conjunctivitis, lacrimation, gasping, sneezing, watery eyes, severe tracheal rales

and cough (Figure 1a to d). Correspondingly, severe conjunctivitis, lacrimation, sneezing, mild tracheal rales and cough have been reported in 2013 in Egypt (Sediek and Awad, 2014). Additionally, IBV infected birds were depressed, lethargic, reluctant to move and take feed these finding was in agreement with (Terregino et al., 2008)

Main lesions in respiratory tract were reddish streaks ranging from mild to severe, increased concentration of mucin in trachea with accumulation sero-mucusexudates in trachea and bronchi (Figure 1c). These findings are correlated with Terregino et al. (2008) and Sediek (2010). However, lungs were congested, discoloured, infiltrated with mucus leading to pneumonia (Figure 1b) that were similar with previous reports (Sediek, 2010). It has been found that sero-mucus exudates in trachea is due to degeneration of cilia by viropexin enzyme produced by IBV (Ashraf et al., 2010). Similarly, infiltration of inflammatory cells in the lamina propria and submucosa, activation of goblet cells, oedema in the submucosa, epithelial lymphoid infiltration, epithelial hyperplasia in trachea have been reported (Cavanagh and Naqi, 2003; Terregino et al., 2008; Sediek, 2010). IBV severely damaged urogenital system especially renal abnormalities (nephritis, pale and enlarged kidneys).

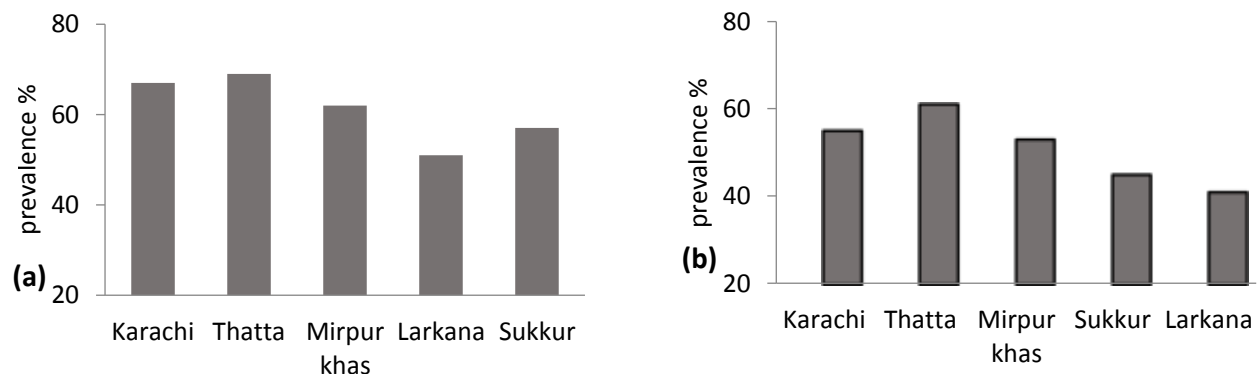


Figure 3. Prevalence % of IBV in broiler birds (a) and layer birds (b) at various districts of Sindh.



Figure 4. (a) Poor quality egg with ruptured shell; (b) Egg yolk distributed in abdominal cavity shown with single arrow head and suppressed shell shown with double arrow. (c) Weak eggs shell with dimples shown by arrow heads. (D) Cracked egg shell.

Moreover, kidney was bulged from renal cavity, inflamed and distended with prominent urates (Figure 2a and b). Correspondingly, Sediek (2005), Sediek and Awad, (2014) and Abdel-Moneim et al. (2002) found that IBV caused severe congestion, pale and congested kidney with prominent urates, peri hepatitis and airsacculitis. On the contrary, nephritis is seen in naturally infected flocks were due to Nephritis Nephrosis Syndrome (NNS) (El-Sisi and Eid-Amal., 2004). The results of the study differ from that of Ashraf et al. (2010) as they reported that IBV isolate 22 does not produce renal lesions in artificial infection.

Results have found that the severity and prevalence percentage of IBV varies with age and type of birds like IBV produce more severe infection in broiler than layer flocks but prevalence percentage is more in layer than broiler that could be due to repeated exposure of layer to same pathogen. Additionally, IBV adversely affect performance of layers and quality of eggs (misshapen, weak shelled, cracked shell, missing of shell with accumulation of yolk in abdomen) (Figure 4a to d). These results are in agreement with previous studies that IBV infected birds have poor laying percentage along with

bad quality eggs (Sediek and Awad, 2014). Interestingly, IBV during growing period appears to have minor effect on the ability of hen to produce eggs of normal quality (Jane et al., 2012).

Hemagglutination assay with trypsin shows that IBV is highly prevalent in layer flocks than broiler, that is, data of layer flocks showed the prevalence in Thatta (69%) to be highest followed by Karachi (67%), Mirpurkhas (62%), Sukkur (57) and Larkana (51%) (Figure 3b) while in broiler flocks at Thatta as 61% and lowest at Sukkur (41%) while at Karachi and Larkana was 55 and 45%, respectively (Figure 3a). Similarly Uddin et al. (2016) found through RT-PCR that IBV was higher in broilers (62.5%) and lower in layer breeders (52.94%).

A total of 90 randomly selected samples were subjected to ELISA, and found IBV strong positive 4/90 (4.44%), positive 46/90 (11.11%), weak positive 33/90 (36.66%) and negative 7/90 (7.77%) (Figure 5). The Findings of this study correlated with that of Hadipour et al. (2011) which showed that infectious bronchitis virus is highly prevalent in poultry with broiler as 64%, layer as 53% and broiler breeder flocks as 54.54% leading to heavy economic losses.

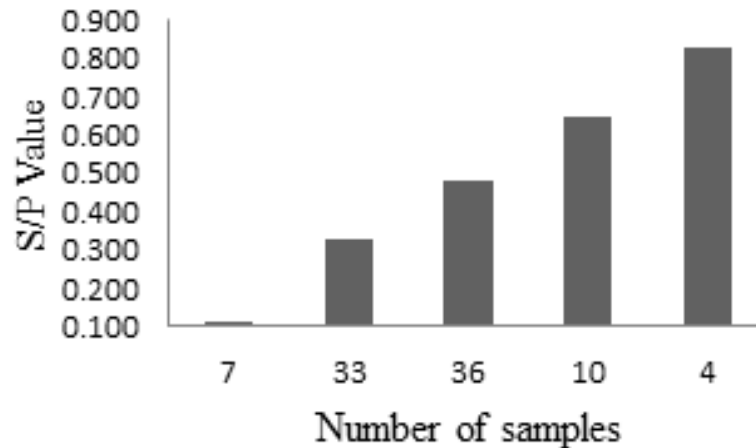


Figure 5. Detection of IBV through ELISA. Samples with S/P value ≥ 0.2 were considered as positive while samples with S/P value ≤ 0.2 were considered as negative

Conclusion

Infectious bronchitis virus is an acute and highly contagious disease of poultry. It causes respiratory distress, heavy morbidity and mortality. Prevalence of IBV is slightly higher in layers (61.2%) than broilers (52 %). Therefore, studies must be conducted on the prevalence, isolation and mechanism of infection and preparation of vaccine from local isolates in-order to prevent economic losses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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