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RESEARCH ARTICLE

Sero-epidemiology and Pathology of Infectious Bronchitis in Commercial Poultry from Faisalabad Division

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ABSTRACT

Infectious bronchitis (IB) is an economically important disease with worldwide distribution. Information is available about the presence of infectious bronchitis virus in commercial poultry in different geographical regions of Pakistan, however no comprehensive information is available about status of infectious bronchitis in commercial poultry. For this purpose, current study was designed for the sero-prevalence of Infectious Bronchitis Virus (IBV) from different commercial poultry farms in Faisalabad division and its adjoining areas. Samples were collected at different age groups and different breeds. Serological detection was carried out by two different means Hemagglutination Inhibition (HI) and Enzyme Linked Immunosorbent Assay (ELISA). In HI, 346 samples from 56 broilers farms were tested for IB and from these 58 (16.8%) samples were positive for IBV tested against 4/91 antigen and 133 (38.4%) samples were positive tested against M-41 antigen. Total of 388 layers samples from 61 layer farms were tested for IB and from these 68 (17.5%) samples were found positive for IBV tested against 4/91 antigen and 184 (47.42%) samples were positive tested against M-41 antigen. In case of ELISA, 1843 samples of layer were processed and 98.48% were found positive, while in case of broilers, from 346 samples, 148 (42.80%) samples were found positive. Grossly hemorrhagic trachea and swollen kidneys were consistent gross findings in all the seropositive birds. Histopathology results showed that there was sloughing of tracheal epithelium and loss of cilia. Renal parenchyma was showing moderate to severe congestion and moderate degree of acute tubular necrosis. The results concluded that there is strong presence and low protection of IB in broiler birds and due to this there is continuous emergence and rise in the disease outbreaks in spite of vaccination. Therefore, proper vaccination schedule with specific vaccinal strains should be followed in broilers and layers to control infectious bronchitis in commercial poultry.

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INTRODUCTION

Infectious Bronchitis (IB) represents an acute disease that is highly contagious which affects the commercial poultry worldwide. The etiological agent is avian corona virus which belongs to genus *Gammacoronavirus*, family *Coronaviridae* and subfamily *Coronavirinae* Valastro *et al*., 2016). IBV viral genome is about 27.6 kb and contains 5' and 3' untranslated regions and contains 3 major structural proteins and one of them is Spike (S) glycoprotein which is highly variable and further it is divided into N terminal S1 and C terminal S2 glycoproteins and 3' un-translated regions and has three major virus-encoded structural proteins (Abro *et al*., 2012).

IBV is not limited to one age group, but it targets chicken of all ages. It affects and multiplies in the upper respiratory tract, epithelial cell of kidneys. It also affects the gut and oviducts thus resulting in poor performance, low egg production, deteriorated egg quality and there may be chances of secondary bacterial infections as susceptibility to secondary pathogens will be more and this can cause condemnations of carcass (Fernando *et al*., 2017).

Up till now multiple genotypes and serotypes have been identified worldwide and due to these continuous

discoveries, these serotypes of IBV give meager or sometimes no cross protection against other serotypes of same group. About 50 serotypes of IBV have been identified up till now and due to the nature of RNA virus (single stranded, positive sense) it undergoes mutation continuously and new variants emerge time to time which are antigenically and pathogenetically different from each other (Cavanagh and Naqi, 2003). The distribution of the serotypes is worldwide in poultry industry (Simmonds, 2015).

The genotype D274 emerged in Western European countries and was the most common type of IBV in the early and mid of 1980s (Worthington *et al.*, 2008). Another genotype of IBV 4/91 that was also known as 793/B was reported for the first time in 1991 in British. This variant was the most dominant in Europe. Different serological studies pertaining to 793/B in broiler and layer chickens revealed the high incidence worldwide (Cook *et al.*,1996).

Pakistan has huge commercial poultry setup and has major role in providing economical source of quality protein and livelihood to the people, but there is always a setback when there is prevalence of a certain disease and one of the important diseases that affect the poultry is IB that poses serious threat and economic loses to poultry production. IBV is prevalent worldwide and also present in Pakistan (Ahmed *et al*., 2007).

Due to inability of the virus strains to give cross protection to field IBV strains, it poses a serious to Pakistan poultry industry which is continuously facing economical pressure. In Pakistan there are limited number of diagnostic laboratories which can confirm the actual presence of the virus and diagnosis is only made by necropsy. Massachusetts-41 (M-41) strain of IBV vaccine is commonly practiced but problems still exist after the vaccination and by serological detection the disease prevalence is still reported in the vaccinated flocks.

However, certain research from the past have indicated that the Massachusetts type classical IBV strains and some of the European variations are still in circulation (Ahmed *et al*., 2007). On the other hand, the regional failure of various IBV vaccines, including those against the Mass-41, 4/91, D-274, and D-1466 strains, repeatedly suggests the presence of certain IBV variants that have not yet been discovered (Bhuiyan *et al*., 2021). As previously mentioned, very few researches have been carried out in Pakistan to the best of the authors' knowledge. For the prevention of infectious bronchitis, many vaccinations are administered, although cross protection is not guaranteed. For successful preventive and control strategies, it's crucial to estimate the seroprevalence of IB. Therefore, the goal of this study is to ascertain the serological prevalence of the variants circulating in layers and broilers.

MATERIALS AND METHODS

Study design

Study area: Samples were collected from the Faisalabad division which includes Faisalabad, Jhang, and Toba Tek Singh districts. Samples were collected by simple random sampling technique from different farms of these areas.

Sample Collection and Storage: Blood was taken from wing vein and stored in vacutainers (no EDTA) for the separation of serum which was stored at -20°C until further processing. In case of broiler birds 346 samples were collected from 56 farms and 388 samples were collected from 61 farms from layer birds.

Hemagglutination inhibition (HI) test: Serum samples were subjected to HI test for the quantification of antibodies against infectious bronchitis virus in serum samples (Swayne *et al*., 1998). For this purpose, two strains M-41 and 4/91 coated antigens were used from Gezondheidsdeinstvoor Dieren B.V. (GD), Animal Health Service, Deventer, the Netherlands. Titers of IB against two antigens (M-41 and 4/91) were simultaneously determined on the same samples. In broiler birds 346 samples were tested from 56 farms and 388 samples were tested from 61 farms from layer birds.

Enzyme linked Immunosorbent Assay (ELISA): A sum of 2189 serum samples were obtained from 122 commercial poultry farms and detected by indirect ELISA kit for IBV (IDEXX Laboratories, USA). The cut-off value was taken as 396 (i.e. ≥396= positive sample) as per the recommendations of the kit manufacturer (IDEXX, USA).

Gross and Histopathology: A total of 50 Samples (Trachea, Kidney and Lungs) were selected on the basis of clinical signs and having lesions of IB and were preserved in 10% neutral buffered formalin. Fixation, blocking, sectioning and staining was done to check different changes at micro level (Bancroft and Gamble, 2008).

Data Analysis and Statistics: The geometric mean titer (GMT) was determined by averaging the well numbers indicating HI activity from each flock's serum samples. The GMT for a given flock against a specific IBV antigen was then provided by comparing this well number average to the GMT values in the Brug's table. Optical Density (OD) value was measured in case of ELISA and titers values were recorded through xChek software (version 3.3) and data (S.D) was analyzed through Microsoft excel statistical analysis software.

RESULTS

Hemagglutination Inhibition Assay (HI) titers results of broilers against M-41 antigen among different age groups: Hemagglutination Inhibition Assay (HI) titers results of total samples were 38.4 % in broilers and 47.42 % in layers as shown in Table 1. Hemagglutination Inhibition Assay (HI) titers results of broiler birds has been shown in the Table 2. In 1st week age group, from 30 samples, 66.66% samples were positive. In 2nd week age group, from 73 samples, 28.76% samples were positive. In 3rdweek age group, from 129 samples, 49.61% samples were positive. In 4th week age group, from 62 samples, 22.58% samples were positive. In 5th week age group, from 52 samples, 26.9% samples were positive

Hemagglutination Inhibition Assay (HI) titers of layers against the M-41 antigen among different age groups: Hemagglutination Inhibition Assay (HI) titers results of layer birds has been shown in the Table 3,1-10-weeks, age group, from 60 samples, 33.33% samples were positive. In 11-20 weeks age group, from 69 samples, 33.33% samples

were positive. In 21-30 weeks, age group, from 68 samples, 63.2% samples were positive. In 31-40 week, age group, from 68 samples, 30.88% samples were positive. In 41-50 weeks, age group, from 60 samples, 45% samples were positive. In 51-60 weeks, age group, from 63 samples, 23.8% samples were positive.

Hemagglutination Inhibition Assay (HI) titers results of broilers against 4/91 antigen among different age groups: Hemagglutination Inhibition Assay (HI) titers results of broiler birds has been shown in the Fig. 1. In 1st week age group, from total 30 samples, 12 (40%) samples were positive for antibodies against 4/91. In 2nd week age group, from 73 samples, 16 (21.91%) samples were positive for antibodies against 4/91. In 3rd week age group, from 129 samples, 20 (15.50%) samples were positive for antibodies against 4/91. In 4th week age group, from 62 samples, 8 (12.90%) samples were positive for antibodies against 4/91. In 5 week age group, from 52 samples, 2 (3.8%) samples were positive for antibodies against 4/91.

Hemagglutination Inhibition Assay (HI) titers results of layers against 4/91 antigen among different age groups: Overall 16.8 samples were positive against 4/91 in broilers and 17.5% in layers as shown in Table 4. Hemagglutination Inhibition Assay (HI) titers results of layer birds has been shown in the Fig. 2. In 1-10 weeks, age group, from 60 samples, 6 (10%) samples were positive for antibodies against 4/91. In 11-20 weeks, age group, from 69 samples, 8 (11.5%) samples were positive for antibodies against 4/91. In 21-30 weeks age group, from 16 samples (23.52%) samples were positive for antibodies against 4/91. In 31-40 week age group, from 68 samples, 14 (20.58%) samples were positive for antibodies against 4/91. In 41-50 weeks age group, from 60, 13 (21.66%) samples were positive for antibodies against 4/91. In 51-60 weeks, age group, from 63, 11 (17.46%) samples were positive for antibodies against 4/91.

ELISA titers of broiler samples according to different age groups (Week Wise): ELISA titers of total samples are shown in Table 5, while broiler samples according to different age groups has been shown in Table 6. In 1st week age group, from 30 samples, 27 (90%) samples were positive for antibodies against IBV. In 2nd week age group, from 73 samples, 19 (26%) samples were positive for antibodies against IBV. In 3rd week age group, from 129 samples, 68 (52.7%) samples were positive for antibodies against IBV. In 4th week age group, from 62 samples, 17 (27.4%) samples were positive for antibodies against IBV. In 5th week age group, from 52 samples, 17 (32.7%) samples were positive for antibodies against IBV.

ELISA titers of layer samples according to different age groups (Week Wise): ELISA titers of layer samples according to different age groups has been shown in Table 7. In 1-10 weeks, age group, from 225 samples, 223 (99.11%) samples were positive. In 11-20 weeks, age group, from 235 samples, 231 (98.2%) samples were positive. In 21-30 week, from 229 samples age group,226 (98.68%) samples were positive. In 31-40 week, age group, from 226 samples, 222 (98.2%) samples were positive. In 41-50 week, age group, from 345 samples, 338 (97.97%) samples were. In 51-60 week, age group, from 176 samples 172 (97.7%) samples were positive. In 61-70 week, age group, from 251 samples, 250 (99.60%) samples were positive. In 71-80 week age group, from 156 samples, 153 (98.07%) samples were positive.

Table 1: Hemagglutination Inhibition Assay (HI) results of total samples collected against the strain M-41 antigen in Division Faisalabad

Bird type	No. of Samples	Positive	Negative	% positive
Broiler	346	133	213	38.4
Layer	388	184	204	47.42

Table 2: HI results of broiler samples collected against the strain M-41 antigen in Division Faisalabad according to age groups

Age of birds (weeks)	Total collected	Positive	% positive	Means ±SD	GMT
1	30	20	66.66	6.9±1.24	119
2	73	21	28.76	5.3±2.32	39
3	129	64	49.61	6.28±2.30	73
4	62	14	22.58	5.6±1.68	48
5	52	14	26.9	4.8±2.1	27

Table 3: HI results (Means ±SD) of layer samples collected from different age group against M-41 antigen in Division Faisalabad

Age (weeks)	Total collected	Positive	% positive	Means ±SD	GMT
1-10	60	20	33.33	4.86±2.07	27
11-20	69	23	33.33	3.85±2.43	13
21-30	68	43	63.2	7.50±1.82	181
31-40	68	21	30.88	5.11±2.14	34
41-50	60	27	45	5.23±2.14	36
51-60	63	15	23.80	3.93±1.77	14

Table 4: Hemagglutination Inhibition Assay (HI) results of total samples collected against 4/91 antigen in Division Faisalabad

Bird Type	No. of Samples	Positive	Negative	% positive
Broilers	346	58	288	16.8
Layers	388	68	320	17.5

Table 5: IBV seroprevalence of total birds estimated by Enzyme Linked Immunosorbent Assay (ELISA) collected in Faisalabad Division

	Total collected	Positive	Negative	Percentage positive	Titer range
Broiler	346	148	198	42.8	1-7065
Layer	1843	1815	28	98.48	1-58725

Table 6: IBV seroprevalence of broiler birds (Mean ± SD) of broiler bird samples estimated by Enzyme Linked Immunosorbent Assay (ELISA) collected in Faisalabad Division

Age (weeks)	Total collected	Positive	% positive	Means ±SD
1	30	27	90.0	2987.5±1762.3
2	73	19	26.0	349±216
3	129	68	52.7	1819.6±2122.9
4	62	17	27.4	324.54±238.1
5	52	17	32.7	1134.6±1810.4

Table 7: IBV seroprevalence of layer birds (Means ±SD) estimated by Enzyme Linked Immunosorbent Assay (ELISA) collected in Faisalabad Division

Age (weeks)	Total collected	Positive	% positive	Means ±SD
1-10	225	223	99.11	2291±631.74
11-20	235	231	98.2	2223.14±848.27
21-30	229	226	98.68	2489.22±845.32
31-40	226	222	98.23	3018.61±2063.52
41-50	345	338	97.97	3330.52±2485.17
51-60	176	172	97.72	2925.19±2131
61-70	251	250	99.60	2591.08±1508.28
71-80	156	153	99.60	2585.86±2652.66

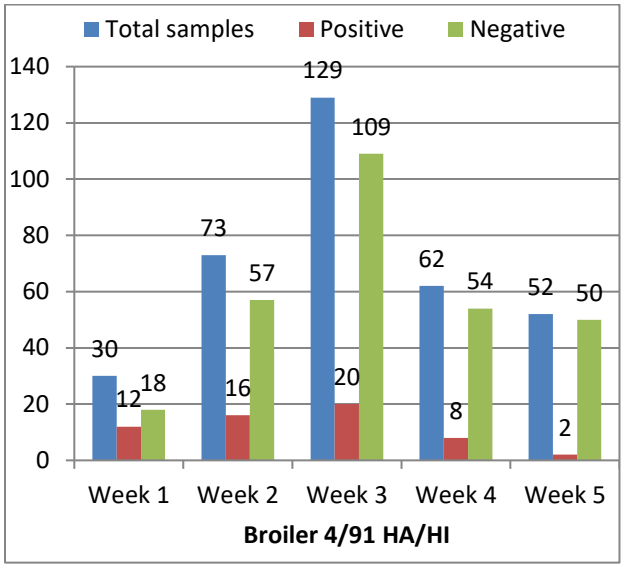

Fig. 1: Graphical presentation of Hemagglutination Inhibition Assay (HI) results of broiler samples collected against 4/91 antigen in Division Faisalabad according to age groups.

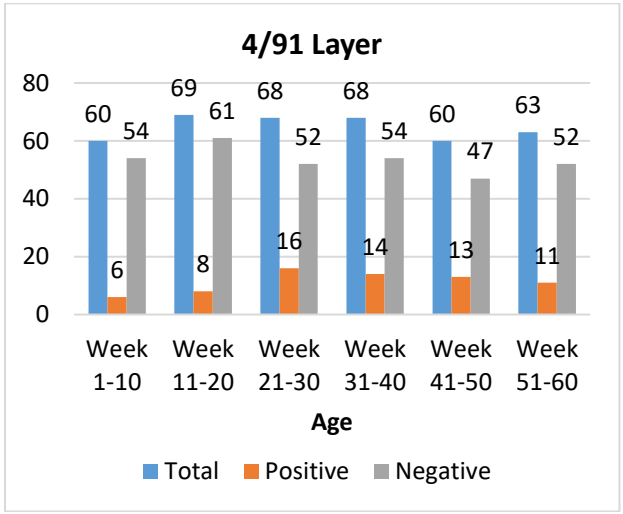

Fig. 2: Graphical presentation of HA/HI results of Layer samples collected against 4/91 antigen in Division Faisalabad according to age groups.

Gross lesions: At necropsy the birds were showing moderate to severe degree of hemorrhages in the trachea. hemorrhagic tracheitis was a consistent finding. Kidneys were bulging out from bony socket and showing deposition of urates. Kidneys were also anemic in appearance. In some birds mild degree of airsacculitis was present having cheesy material. Lungs were congested in some birds.

Histopathology: The most prominent histopathological lesions observed in IBV infected birds in renal parenchyma was tubular necrosis, congestion and hemorrhages (Fig. 3 and 4). In case of trachea infiltration of inflammatory cells, loss of cilia, necrotic changes, congestion and epithelial hyperplasia were prominent histopathological findings (Fig. 5). The pulmonary parenchyma congestion, pneumonic changes and fibrinous pneumonia. The hepatic parenchyma was showing mild to moderate degree of necrotic changes. Mostly individual cell necrosis was present. Mild degree of vacuolar degeneration was present however vacuoles were hazy in appearance.

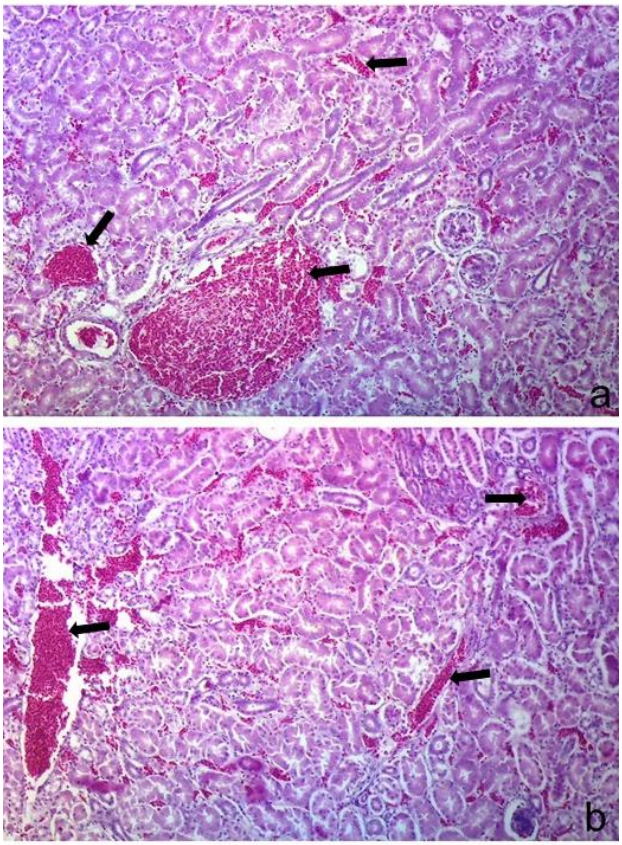

Fig. 3: Photomicrograph of kidney of IBV infected birds having renal congestion and hemorrhages indicated by black arrows both in figure a & b (H & E Staining 100X)

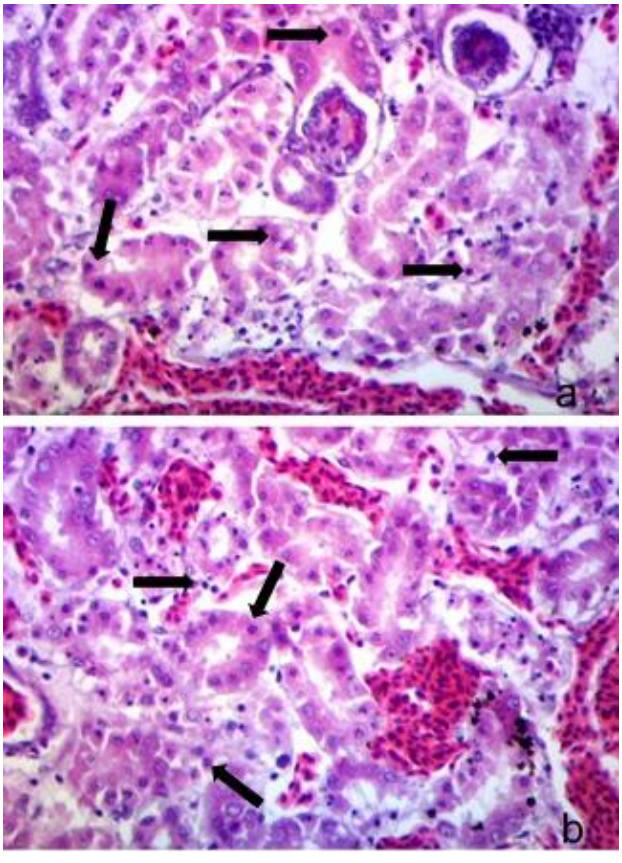

Fig. 4: Photomicrograph of kidney of IBV positive birds showing tubular necrosis indicated by black arrows in both figures a & b (H & E Staining 400X).

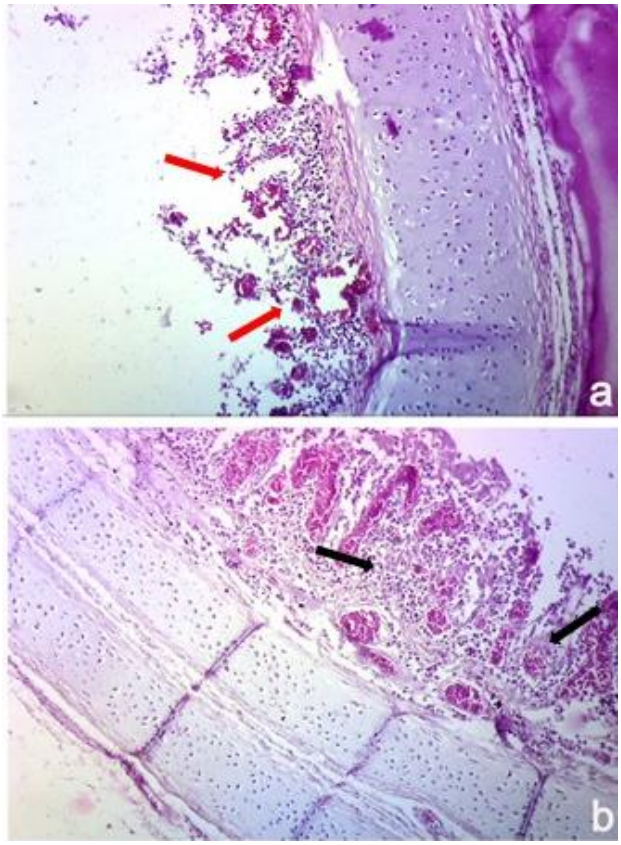

Fig. 5: Photomicrograph of trachea showing necrotic changes with lymphocytic infiltration (red arrow) in both figures a while figure b showing and congestion epithelial hyperplasia and metaplasia (H & E Staining 100X).

DISCUSSION

Infectious bronchitis is a coronavirus that causes severe respiratory and renal disease in commercial broiler and layer birds leading to high mortality and economic losses. The virus is of high mutating nature and causes disease even in vaccinated flocks. There was a need for an epidemiological study in order to find out the prevalence of IBV in the commercial poultry sector.

A total of 346 samples of broilers and 388 samples from layers were collected for seroprevalence through HI. Hemagglutination Inhibition (HI) against M-41 showed slightly higher prevalence of IBV in layers (47.42%) than in broilers (38.4%) in our studies. IBV prevalence in layer flocks was significantly higher than that in broiler flocks and further showed a larger proportion of samples with high levels of antibodies. This could be due to that layer birds are long living birds receiving repeated doses of vaccines than broilers and subsequently becoming re-infected in the absence of suitable control method (Ayim-Akonor *et al*., 2018). Similar to our findings Andreopoulou *et al*., 2019) detected higher rates (83.96%) of IBV in layer and broilers flocks in Greece respectively. Research shown by Worthington *et al*. (2008) had similar rates (57%) to our research of commercial poultry in Spain. Titers against M-41 in broilers in 1st week of age group were highest (66.66%) which might be maternally derived antibodies (MDA), while the lowest was found in 4th week of age group (22.58%). In layers the highest prevalence was seen in 21-30 week of age (63.2%) as it is young age having maximum immunization, while the lowest prevalence was seen in 31-40 week of age group (30.88%) may be due to production stress and exposure of the birds to different filed challenges. The previously available research (Ahmed *et al*., 2007) which conducted an IBV seroconversion analysis in poultry industry reinforces our analysis. The data found that 88% of the flocks had Mass 41 antibodies that were seropositive while 8% of the flocks were IB 4/91 strain positive. Rafique *et al*. (2018) also performed HI test on different strains of IBV (M-41, 4/91, etc.), HI against 4/91 showed higher prevalence in layers (17.5%) than in broilers (16.8%). In broilers according to age the highest prevalence was seen in 1st week of age group (40%) and the lowest was seen in 5th week of age (3%). In layers 21-30 week of age group showed the highest prevalence (23.52%) while the lowest prevalence was observed in 1-10 week of age group (10%). Rahim *et al*. (2018) noted that seroconversion was seen against IBV serotypes H-120 (GMT=113) and 4/91 (GMT=106) at various poultry breeder farms in Multan and Sihala (Islamabad), whereas seroconversion against IBV serotypes M-41 was seen at Jumma Bazar broilers poultry breeder farms in Abbottabad (GMT=92) and Chakwal (GMT=98).

The IBV strain M-41 was found to be highest in Pakistan (Muneer *et al*., 1987) which is supporting our findings. Hussain *et al*. (2005) reported similar findings, indicating that the IB seroprevalence was much greater in birds aged 1-2 week (5.36%), with 3-4 and 5-week-old chicks having the lowest frequency.as the age increases IBV becomes resistant in chicken resistance and decreased mortality is seen (Smith *et al*., 1985; Albassam *et al*., 1986).

Meager information is available about occurrence of various IBV serotypes and their variants in Pakistan in available published data/literatures/publications. In a different study Muneer *et al*. (1999) conducted experiments in local (desi) birds and IBV antibodies have been identified for different serotypes like Massachustts-41, Arkansas, JMK, D-274 and D-1466. The study concluded that antibodies in non-vaccinated flocks have a greater prevalence in areas where poultry farms are situated in clusters or in multiple ages groups.

A commercial ELISA was used to detect IBV antibodies. A total of 2189 samples were analyzed through indirect ELISA method. Current results showed strong positive IB titers in layers and broilers. This study's results were similar to those in Hadipour *et al*. (2011) that revealed a high prevalence of infectious bronchitis in chickens with 64 percent broiler and 53 percent layer, resulting in severe financial losses. In our studies there was high seroprevalence in broilers and layers. Alam *et al*. (2003) and Biswas (2004), who showed seroprevalence of IBV 92.06% and 77.83%, respectively, reported similar findings. Broilers (42.8%) and layer birds (98.48%) had the highest prevalence rates. Kanwal *et al*. (2018) reported a similar finding through ELISA in Pakistan showed that 84.4% of samples tested positive for IBV whereas 14.6% tested negative and it concluded that the Pakistani province of Sindh has a significant prevalence of IBV. It would be important to note if the findings of Darbyshire & Peters (1984) regarding the adverse association between the degree of serological reactions and the extent of (homologous) bird safety still extend in this research in regard to the heterologous challenge following vaccination

with H120, if there were no cross protected trials in this study for broiler.

Our study showed that broiler in week 1 had the highest titers this result was in line with that of Mungadi *et al*. (2015), who observed that there was a substantial difference in frequency between developing chickens and adult chickens. This result agreed with Cavanagh and Gelb (2008) which claimed that while all age groups of chicks are susceptible to IBV, younger chicks are more vulnerable than older ones. In layers the highest titer was observed in week 41-50. In the domestic birds of Mexico, in the year 2000 there were 56.5% ELISA based IBV seroprevalence (Gutierrez-Ruiz *et al*., 2000), 50% of the 236 non-vaccinated birds in UK were IBV seroconversion (Meulemans *et al*., 2001) and 92.5% of the 40 fancy poultry stocks in Switzerland (Wunderwald and Hoop, 2002), respectively.

The above analysis reinforces our research, since the conventional results are concerning the distribution of untyped IBVs in the field that require additional research to type such IBV isolates out of the domestic, the wild and market poultry. In the first place, a routine detection and surveillance service for IBV will be defined. With respect to the origin of the spreading IBV varieties in the region, it seems that in countries like Pakistan the borders security system is free to exotic and migratory birds. Indeed, very little structured data exist in most of the countries of the region concerning various forms of avian pathogens, like IBV. What makes the emergence of types of birds in a region very difficult to detect, making it difficult for the control plan to be implemented in order to counteract those preliminary stages of infections.

The histopathological analysis of trachea, lungs and kidneys was carried out in the study. In renal parenchyma tubular necrosis, congestion and hemorrhages. Similar changes were observed by Jolly (2004) who reported lymphocytic infiltration in the kidneys of birds that were infected with T-strain. In trachea infiltration of inflammatory cells, loss of cilia, necrotic changes, congestion and epithelial hyperplasia were prominent histopathological findings. These similar changes were observed by Booromand *et al*. (2012) in which there was congestion and oedema of tracheal mucosa. Sometimes, hyperplasia was also observed. Chousalkar *et al*. (2007) also reported similar changes in trachea in which there were clear histopathological changes as of our findings. The lungs of clinically sick birds showed mild to moderate degree of congestion in pulmonary parenchyma. These changes were in line with Lisowska *et al*. (2021) that reported moderate to severe congestion in the lungs, with the presence of inflammatory cell infiltration and hemorrhage into the lungs.

Conclusions: This study is the infectious bronchitis virus is prevalent in the broiler and layer flocks of Faisalabad Division. The study also finds out that there is a need for establishment of better monitoring strategies and facilities in order to combat the threat and contain it as much as possible. In the end, there is a need for a thorough molecular epidemiological study along with phylogenetic analysis of the isolates in order to identify any new strains that might be present in Pakistan.

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Authors contribution: MKS designed and planned the study, AF & MKS executed the field study and sample collections, AF, MKS & STG analysed the samples in the laboratory. MKS, MMG and HI participated in write up of manuscript.

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