

## **Clinical evaluation of SPF chickens infected with 793/B serotype of Infectious Bronchitis virus**

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### **ABSTRACT**

Avian infectious bronchitis virus (IBV) is one of the most important viral diseases in almost all countries with intensive poultry industry. This disease characterized primarily by respiratory signs, but some IBV serotypes may also cause other signs such as the involvement of intestine and kidney. The aim of this study was to characterize the Clinical signs and gross lesions of 793/B serotype of avian infectious bronchitis virus in experimentally infected SPF chickens. Forty two one-day-old SPF chicks were divided randomly into two groups (21chicks each group). At the age of 12 days old the chicks in group1 were inoculated intra-ocularly with  $10^3$ EID<sub>50</sub> of the 793/B isolate, and the group2 was as control. Chickens in each groups was evaluated from 1 to 14 days post inoculation. The results of this study indicated that the 793/B serotype of IBV cannot cause mortality, and only slight clinical signs such as gasping and depression were seen in infected chickens. Gross lesions indicated this serotype only induces slight lesions in trachea, but in kidney and bursa of fabricius the lesions were severe. These results demonstrated that 793/B serotype cannot cause mortality, sever clinical signs or gross lesions in infected chickens, but its effects on lymphoid tissues should be further studied.

**Keywords:** Infectious Bronchitis, 793/B serotype, Clinical Signs, SPF chickens, Serology

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### **INTRODUCTION**

Infectious bronchitis (IB) is an acute, highly contagious, and economically important viral disease of commercial chickens occurring at all ages, which is caused by infectious bronchitis virus [10]. Infectious bronchitis virus (IBV) is a member of *Coronaviridae* family and genus *Gammacoronavirus*, with more than 26 serotypes [13, 18]. Although IBVs are primarily causes respiratory disease [16], but some strains of IBVs also infect non-respiratory tissues including reproductive tissues [14, 33], kidney [12, 36], and alimentary tract [37]. In field cases IBV characterized by tracheal rales, coughing, sneezing and kidney swelling.

The severity of clinical signs in chickens related to their age, immunity against IBV, nutritional status, and concurrent infections [4, 26]. In Iranian chicken flocks IBV was first reported in 1994, and it was confirmed by serological and virus isolation methods, at that time its revealed that the Massachusetts serotype is a major serotype

of IBV [1]. Later, several Iranian researchers identified the 793/B serotype [24, 30, 34], turned out to be one of the predominant circulating types of IBV in Iran [25, 31].

However, this serotype was not further studied and thus, pathologic properties are not well characterized. The aim of the study was to investigate the clinical aspects of 793/B serotype of Infectious Bronchitis virus in SPF chickens as well as clinical signs and gross lesions. Serological responses of chickens after infection were also evaluated challenged chicks.

## MATERIALS AND METHODS

**Virus:** The Iranian isolate IR/773/2001 (793/B serotype) of IBV was used in this study. This strain was isolated from Iran [24]. The titer of virus were expressed as the 50% embryo-infective dose (EID<sub>50</sub>) calculated by the method of Spearman-Kärber [35].

Forty two white Leghorn embryonated chicken eggs were obtained from the specific pathogen free (SPF), Venky's company (Venky's, India), after hatching were divided randomly into two equal groups. They were kept in separate positive pressure isolators at Razi Vaccine and Serum Research Institute, Karaj-Iran.

**Experiments:** At the age of 12 days-old, all birds of the experimental group were inoculated 10<sup>3</sup> EID<sub>50</sub>/0.1 ml of IBV serotype-793/B by eye drop. The other group was kept as a control. After virus challenge, all the chickens were monitored daily for clinical signs and mortality. From 2 to 14 days post inoculation (PI), three chickens from each group were randomly selected and humanely euthanatized and necropsy was performed and gross lesions were recorded.

**Serology:** Serum samples were tested for the presence of antibodies against Infectious Bronchitis using the ELISA test. Commercial IDEXX ELISA kit was used for antibody detection against Infectious Bronchitis virus.

**Statistical analysis:** The results obtained from ELISA test were analyzed by independent t-test, using PASW SPSS software (version 18.0). The t-test was performed at 95% probability and p-value less than 0.05 was considered significant and less than 0.01 was considered as very significant.

## RESULTS AND DISCUSSION

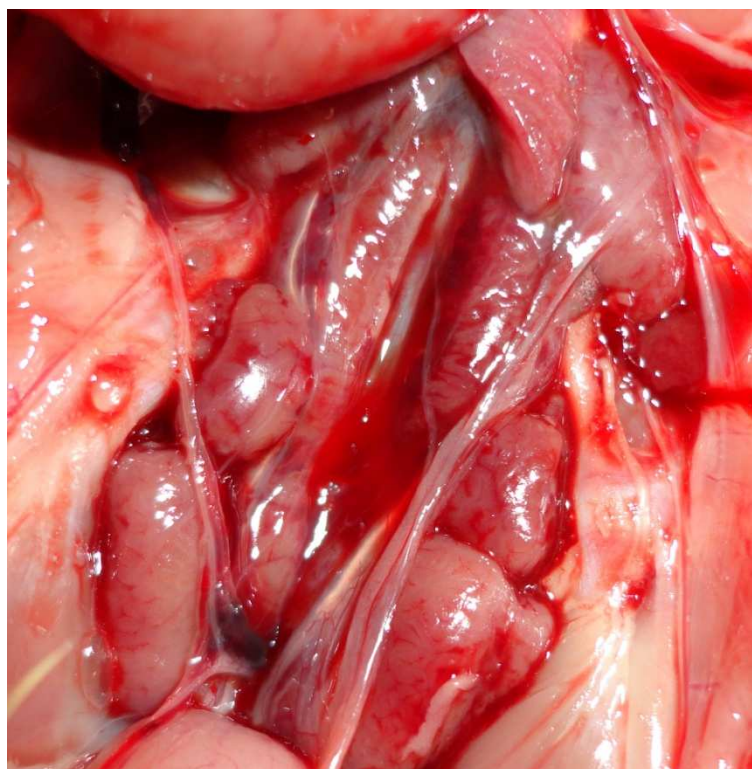
**Clinical Signs;** Some chickens of the infected group showed mild gasping and depression at 2 days PI and immediately recovered from days 4 post inoculation. There was not mortality in any groups during the experiment and there were not any clinical signs or gross lesions in control group.

**Gross Necropsy Findings:** The chickens infected experimentally with 793/B serotype of IBV showed the following lesions. In tracheas only slight hyperemia and serous exudates were seen at day 2 and 4 PI and after that the tracheas were almost normal in gross necropsy. Kidneys were pale and swollen at day 4 PI and it last until 10 day PI, and then the kidneys gradually became normal. The Bursa Fabricius was shrunk at day 6 PI and Thymus was shrunk at day 4 PI in comparison to control group. Other organs also were normal and there were not any gross pathological changes.

**Serological findings:** Antibody titers against Infectious Bronchitis in the serum samples that were collected on days 0, 2, 4, 6, 8, 10, 12, and 14 PI was measured by ELISA test. All serum samples on day 0 and 4 PI were negative to IB, serological results of IB are shown in Table-1, statistical comparison between two groups indicated that from days 4 to 14 there was significant difference between two groups and at days 6 to 14 PI there was very significant difference between groups. There was not any change in antibody titer against IB virus in the control groups.

**Table1: Antibody titers against Infectious bronchitis in experimental groups (Mean±SE)**

Groups	Days Post Inoculation						
	2	4	6	8	10	12	14
<b>IB Infected</b>	110.01±10.54	341.68±50.45	503.57±78.91	891.09±105.09	1482.27±146.57	2054.61±237.57	2431.07±261.83
<b>Control</b>	105.46±8.91	120.15±11.14	117.64±9.82	118.01±12.57	114.76±10.85	124.21±11.94	119.64±10.84
<b>t-test p-value</b>	0.89	0.04	0.01	0.01	0.01	0.01	0.01



**Fig1- kidney, 8 days PI, swelling and abnormality in kidney**

Since 793/B serotype outbreak was reported by several researchers in Iran [24, 30, 34], it's become predominant circulating types of IBV in Iran. Although IBV pathogenesis has been studied by various techniques [5, 11, 21, 22], the present study was conducted to determine the pathological changes due to 793/B serotype of IBV, reported from Iranian poultry flocks [24].

Parsons *et al.* (1992) and Capua *et al.* (1994) indicated that the IBV infection in the respiratory tracts causes characteristic signs, such as gasping, coughing, tracheal rales and nasal discharge. Occasionally, puffy, inflamed eyes and swollen sinuses may be seen [9, 28]. Mc-Martins has reported that the upper respiratory tract is the primary site of IBV infections, after which widely spreads to other organs [23]. Mild clinical signs and limited gross lesions by 793/B serotype in respiratory tracts have been described [5]. Some studies indicated that inoculation of IBV in commercial chickens causes conjunctivitis, excessive lacrimation, oedema, and cellulitis of the periorbital tissues [32], while following challenges broiler chickens with 793/B serotype only mild tracheal rales, coughing, gasping, and swollen kidneys were reported without any mortality [7].

It was reported that the birds inoculated with 793/B serotype had not any clinical signs or mortality [5]. In uncomplicated cases, food consumption and weight gain significantly reduce within 3 days after infection and chickens become depressed [15, 27]. In our study, the only clinical signs were depression and reduced weight gain compared to the control group which is in agreement with previous reports [5, 21, 27, 28], and the results of this study revealed that this serotype cannot cause any mortality, which this results in agreement with Benyeda, et al (2010) and Boroomand, et al (2012) studies, that they reported 793/B serotype IBV had not induces any mortality in experimental situations.

Some strains of IBVs are nephropathogenic and has a tropism to respiratory tissues and kidney, but the lesions which they cause are more evident in the kidney [2, 6, 8, 17, 29]. Several reports confirmed this serotype of virus had tropism to kidney and produce lesions in chickens [2, 5, 7]. Our results also indicated that the 793/B serotypes have more tropism to kidneys and lesions in kidneys more prominent and last longer.

H52 and H120, IBV from the bursa fabricius of experimentally infected chickens was isolated [3, 20]. Also virus tropism to the trachea, lung, intestine, and bursa of inoculated embryos were reported [19]. In our study the thymus and bursa of fabricius was shrunken and it is in agreement with previous studies and indicates that IBV could cause immunity problems in chickens. It was described that the 793/B serotype is enterotropic and pneumotropic in broiler chickens and also associated with diarrhea [7], but there was no reports of gross or histological changes.

In our study, weight gain was reduced significantly ( $p < 0.05$ ) in comparison to control group during study period. The results of serological examination showed antibody increase in IB infected groups and this results was in agreement with previous studies results [7].

### CONCLUSION

Our results indicate that the 793/B serotype of IBV cannot cause mortality, sever clinical signs or gross lesions in infected chickens, but its effects on Bursa of fabricius could induce lesions of other concurrent infections and expose birds to other bacterial or viral disease. Further studies are required to confirm the role of birds strain and other pathogens on outcome of this disease.

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