Iranian Journal of Virology 2018;12(2): 13-18
©2018, Iranian Society of Virology

Original Article

Evaluation of the Antibody Titration in Vaccinated Chickens Against Infectious Bronchitis Virus Using Three Commercial ELISA Kits

Mohammad Hossein FallahMehrabadi [1], Seyed Ali Ghafouri [2], Arash Ghalyanchilangeroudi [3,*], Hossein Hosseini [4], Reza khaltabadi Farahani [5], Hossein Maghsoudloo [5], Hamed Abdollahi [5], Zahara Ziafati Kafi [3], Mona Hamedi [3], Leila Aghayian [3]

1. Department of Poultry Diseases, RAZI Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad
3. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, Iran
5. Iranian Veterinary Organization, Tehran, Iran

Abstract

Background and Aims: Infectious bronchitis is an economically important disease, especially in chickens. It causes disorders in the respiratory tract, kidney and reproductive tract of affected birds. The annual losses imposed by the disease are significant in the Iranian poultry industry. The Infectious bronchitis virus has many different serotypes and mutations in its RNA results in the virus variation which makes the control of IB more difficult. The control strategy of IB is based on vaccination and it has been used live and inactivated vaccines. Vaccines against different strains of the virus have been used. Vaccines should be against specific strains in each area. The application of an appropriate ELISA kit which can detect the level of antibody response leads to choosing an effective vaccine.

Materials and Methods: The current study compared antibody response after four vaccination approach and then compared 3 ELISA kits for the detection of antibody rising. A total of 100 SPF chickens were divided into 5 groups. The first group considered as the control and H120-H120, H120-1/96, H120-4/91, and H120-IB88 protocols were conducted for the 2nd, 3rd, 4th, and 5th groups, respectively. The validation of the Proflok, BioChek and IDEXX ELISA kits were evaluated after vaccination.

Results: Significant differences in titers between four vaccination approaches were shown better by Biochek, Idexx and Proflok kits respectively. Also, the highest antibody titration belonged to the 4th group and the highest titration detected by the Proflok kit which had the most sensitivity.

Conclusions: According to our results, it is important to use endemic strains of the IBV for vaccination to have better cross-protection. In this study, as the 3 studied kits had different sensitivity and specificity, different antibody rising was detected.

Keywords: ELISA kit, Infectious bronchitis, Iran, Vaccination protocol.

Introduction

[*]**Corresponding author:** Arash Ghalyanchilangeroudi, Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, Email: arashghalyanchi@gmail.com; ghalyana@ut.ac.ir, Tel: 009861117000.

Infectious bronchitis (IB) is an acute disease which affects chickens of all ages. The causative agent is a virus belonging to the Coronaviridae family and the genus Gamma-coronavirus. Although the virus is primarily infectious for the respiratory system, it can also infect the reproductive tract and some strains may cause nephritis [1]. The virus spreads directly or indirectly via contaminated feed or water (Oie, 2018) and results in economic losses through reproductive disorders in breeders and layers, poor weight gain in broilers, and mortality which can be more than 50% [2].
As recombination occurs in the RNA of the virus, new genotypes are creating and although different countries have overcome many of them, new mutants which haven't any cross-protection, are spreading in the world [3, 4]. A study in 2009 in Tunisia characterized new variants of the viruses using genotyping and serotyping techniques and the variants had 57-78% similarities to the European genotypes [5]. The annual losses imposed by the disease are significant in the Iranian poultry industry, despite mass vaccination, though there isn't any identical control program in the country [6].
In a study in 2004, IBV was detected in 42.8% of samples from different provinces of Iran [7]. Mahzounieh et al. in 2006 found 85.3% of chickens in villages of Iran had high titers of antibody against IBV without any clinical signs [8]. Similar to many other countries, the viral genotypes are changing in Iran and it makes the vaccination programs more difficult [9, 10]. During 1999-2004, 52.7% of Iranian flocks were positive for 793/B serotype of IB virus (IBV) [11].
While during 2014-2015, Najafi et al. demonstrated Massachusetts (Mass), 793/B, IS-1494, IS-720, QX,4IR-1, and IR-2 as the most prevalent genotypes in Iran [12]. In 2017, Shokri et al. detected 793/B, variant 2 and QX in the backyard flocks of Iran [13] According to the high prevalence of several variants of IB throughout the world (Oie, 2018), implementation of control programs of the disease is essential. The control strategy of IB is based on vaccination and it has been used live and inactivated vaccines. Attenuated live vaccines such as H120 and Ma5 which are using in broilers and pullets are expensive, though they have better immunity (Oie, 2018). Killed vaccines are using for layers and breeders [2]. In order to achieve the protection of chickens from IB through vaccination, it is necessary to choose an appropriate vaccine against endemic strains. What commonly has been used for the best immunity against several genotypes is the combination of vaccines such as H120, Ma5, 1/96, IB88 and 4/91. In the United Kingdom, a combination of Ma5 and 4/91 is one of the most vaccination programs in broilers [14].
Several types of research have evaluated the efficacy of the vaccine combination against different genotypes [15, 16] but there are a few studies about the antibody titer of different vaccines which are using in poultry farms. Moreover, the differences in antibody rising of different commercial ELISA kits have not been evaluated following vaccination. The present study evaluated the antibody titration after a combination of four vaccines against infectious bronchitis. Then it compared 3 ELISA kits for their antibody response.

Methods

The study design. A total of 100 one day- old SPF chickens which have no maternal antibody against IB, were grouped into 5 of 20 birds. The first one was unvaccinated (the control group) and the others were vaccinated by eye-drop as follows:
At the age of 1 day- old, all birds except the control group received H120 (CEVA) and at the age of 14 day-olds, chickens in the group 2 again were vaccinated with H120 (CEVA). The group 3, 4 and 5 were vaccinated with 1/96 (CEVA), 4/91 (Intervet) and IB88 (Merial) respectively. On the 35th day, blood samples were collected from all the groups. Then ELISA was conducted by the IDEXX (Westbrook, Main, USA), BioChek (Gouda, Holland) and Proflok (Synbiotic, Edison, NJ, USA) kits and the titers of antibody in the groups were evaluated by each ELISA kits according to the manufacturer manual.

Table 1. The antibody titers of the group according to the ELISA kits. Non-similar small Latin letter that there is a significant difference ($p<0.05$).

The studied groups	The ELISA kits		
	Proflok	**BioChek**	**IDEXX**
	The mean antibody titers	**The mean antibody titers**	**The mean antibody titers**
H120-4/91	6073.26±4016.8 a	2891.55±1395.13a	2162±1349.66a
H120-1/96	6061.16±3944.64 ab	2878.31 ± 1371.82ab	1776.84±1359.79ab
H120-IB88	4111.38±2836.53abc	1874.48±831.32c	1447.07±767.03abc
H120-H120	4385.55±2218.95 abcd	1401.41±559.96cd	892.33±663.52bcd
The control group	0e	0e	0e

Statistical analysis. The comparison of the rise in antibody titer of each vaccine and the mean of antibody response with each commercial kit was evaluated using one-way ANOVA. The differences were considered significant at $P< 0.05$.

Results

All the vaccinated groups had a significant rise in the antibody titer compared to the control group (Table 1.). The serum from the 4th group which received 4/91 vaccine in their 14-day olds, had the most antibody response. There was no significant difference between vaccination approaches in the Proflok kit (Fig. 1c. and Table 1). The Proflok kit showed the highest antibody titer compared to the other (Table 1). There was no significant difference in the Biochek kit between the 3rd group and

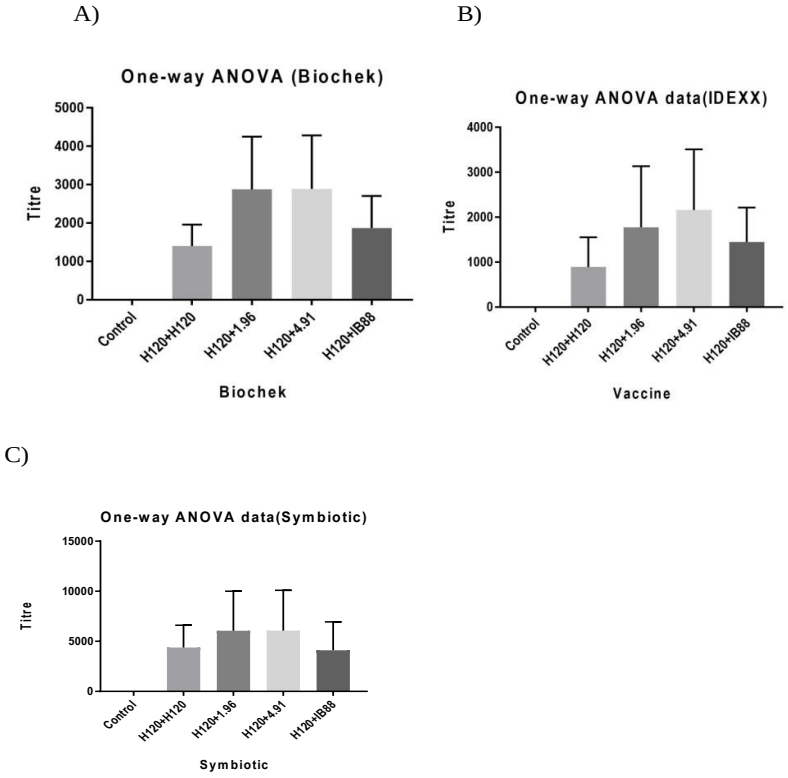


Fig. 1. The mean antibody titer of vaccines in the 5 groups. Significant antibody rising is seen in all the vaccinated chickens compared to the unvaccinated group. (The BioChek kit. A, The IDEXX kit. B, The Symbiotic (Proflok) kit C).

the 4th group, but this difference was significant between the 4th group and the other groups (Fig. 1a. and Table 1). Although the antibody titer of the BioChek kit was higher than the IDEXX. There was a significant difference in the Idexx kit between the 2nd group and the 4th group, but this difference was no significant between the 4th group and the other groups (Fig. 1b. and Table 1).

Discussion

To our knowledge, it is the first study that evaluates the antibody titers of different vaccines and simultaneously, validates the obtained titers according to different commercial ELISA kits. We compared 4 approaches for vaccination against the Infectious bronchitis virus and according to our results, the combination of H120 and 4/91 vaccines had the best antibody titer.

Moreover, this difference of the titer was significant compared to the H120-H120 and H120-IB88 groups. The highest antibody response after the H120-4/91 group was seen in the H120-1/96 group but this difference wasn't significant.

Habibi et al. in 2016 compared the protection of the combination of the H120- H120 and H120- 1/96 vaccines. They found that the best cross-protection was obtained by the use of H120-1/96 combination [17].

In our study, we also demonstrated that the antibody titer of the group vaccinated with H120-1/96 had a significant difference with the H120-IB88 group, though the difference H120-IB88 wasn't significant compared to the second group (H120-H120) with Biochek kit.

It is evident that the combination of H120-1/96 has the best protection against IBV in the world [17]. Our study does not confirm these findings and it was shown that the antibody titers in the chickens vaccinated with H120-4/91 had high titration using all the 3 ELISA kits.

Besides H120 and 1/96 strains, the 4/91 is another common serotypes in Iran [7], so using a protective vaccine against this strain is essential. We determined that the combination of H120-4/91 has the highest antibody rising in the studied chickens. In the EU, the most protective and beneficial vaccine in broiler chickens is 4/91-Ma5 [11]. In addition, it seems that this protocol induces high levels of protection against heterologous IBV types such as D1466 and QX [18]. In the study of Smialek et al. in 2016, the 4/91 strain had a wider spectrum cross-protection and more induced the production of IgA [14].

In the present study, we evaluated the antibody titers of the vaccines in order to have information about their antibody rising to choose an appropriate vaccination protocol in the cases of IBV infection, but as the dominant types of the virus are changing in the world [19] we should evaluate antibody responses against different genotypes of the virus.

Karimi et al. in 2018 implemented the two vaccines H120 and Ma5 against challenging with the QX strain of IBV and they found that none of the vaccines can induce cross-protection against the virus [15]. Hamadan et al. in 2017 reported the most dominant genotypes in Iran including IS-1494 –like IBV, 793/B type, Massachusetts and QX type respectively (Hamadan et al., 2017), so using a vaccination program which has cross-protection against these types may reduce the viral load in our farms. It has been determined that vaccines included 4/91 have effective protection against the QX strain [20] and antibody titers of H120-4/91 in the present study were 2162, 6073.26 and 2891.55 using the IDEXX, Proflok, and BioChek ELISA kits respectively (Table 1.).

Several commercial ELISA kits are available for the detection of the IBV and this test has been used for the monitoring of antibody responses to vaccination against [21] [20]. The necessity of this study came from the most clinicians and technicians issue in the laboratory as well as industry; which was about how to compare these three commercial kits and how each kit can be approximately equivalented. We definitely need to know each vaccines' baseline to figure out vaccination approaches, and in the same time we should know how these vaccines will be tittered individually in SPF chicks. There was no available data to compare these kits; baselines

and vaccination programs in a SPF chick to rely on and achieve a result. In the present study, we demonstrated that Proflok is the most sensitive ELISA kit as it showed significantly higher antibody titers compared to the BioChek and IDEXX in all the vaccinated groups (Table 1). Moreover, the BioChek kit which has more sensitivity than the IDEXX kit, showed more antibody rising compared to the IDEXX. Significant titers differences between four vaccination approaches were shown better by Biochek (Table 1). In poultry, the high specificity of ELISA kits is more important than high sensitivity because we can retrieve low sensitivity by using more blood samples [22]. In our study, although the IDEXX kit showed the lowest level of antibody titer, its high specificity is valuable in the excluding of non-infected birds.

Conclusion

Infectious bronchitis virus needs a monitoring program to evaluate new and dominant serotypes in different regions of Iran. Knowing endemic strains leads to a suitable vaccination strategy in the country. Validation of new protocols of vaccination against new genotypes requires reliable ELISA methods to evaluate antibody titers against the virus. In the present study, we recommend the combination of H120-4/91 for the vaccination of the poultry. According to our results, the differences in sensitivity and specificity of commercial ELISA kits should be considered in the case of evaluation of antibody rising after vaccination.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Cavanagh D. Coronavirus avian infectious bronchitis virus. Vet Res. 2007;38(2):281-297.
2. Jackwood MW. Review of infectious bronchitis virus around the world. Avian Dis. 2012;56(4):634-641.
3. Lin SY, Chen HW. Infectious bronchitis virus variants: molecular analysis and pathogenicity investigation. Int J Mol Sci. 2017;18(10):2030.
4. Wit J, Cook J, Van der Heijden H. Infectious bronchitis virus in Asia, Africa, Australia and Latin America: history, current situation and control measures. Braz J Poult Sci. 2010;12(2):97-106.
5. Bourogâa H, Miled K, Gribaa L, El Behi I, Ghram A. Characterization of new variants of avian infectious bronchitis virus in Tunisia. Avian Dis. 2009;53(3):426-33.
6. Davam H, Ghalyanchi langeroudi A, Masoud Hashemzadeh , Karimi V, Zabihi T, Seger W, et al. Full-length characterization of S1 gene of Iranian QX Avian Infectious Bronchitis virus isolates. 2015. Iran J Virol. 2016;10(4):18-25.
7. Seyfi Abad Shapouri M, Mayahi M, Assasi K, Charkhkar S. A survey of the prevalence of infectious bronchitis virus type 4/91 in Iran. Acta Vet Hung. 2004;52(2):163-166.
8. Mahzounieh M, Karimi I, Bouzari M, Zahraei Salehi T, Iravani S. A serological survey for detection of avian infectious bronchitis virus antibodies in domestic village chickens in Esfahan, central Iran. Iran J Vet Res. 2006;7(2):89-91.
9. De Wit J, Cook JK, Van Der Heijden HM, Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol. 2011;40(3):223-235.
10. Hamadan AM, Ghalyanchi Langeroudi A, Hashemzadeh M, Hosseini H, Vahid Karimi V, Yahyaraeyat R, et al. Genotyping of Avian infectious bronchitis viruses in Iran (2015–2017) reveals domination of IS-1494 like virus. Virus Res. 2017;240:101-106.
11. Bande F, Arshad SS, Omar AR, Hair-Bejo M, Mahmuda A, Nair V. Global distributions and strain diversity of avian infectious bronchitis virus: a review. Anim Health Res Rev. 2017;18(1):70-83.
12. Najafi, H., et al. Pathogenicity study of Iranian genotype of avian infectious bronchitis virus (IR-1). in Veterinary Research Forum. 2017. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
13. Shokri S, Karimi V, Ghalyanchi Langeroudi A, Vasfi Marandi M, Hashamzadeh M, Zabihi-Petroudi T, et al. Seroprevalence and genotyping

of avian infectious bronchitis virus detected from Iranian unvaccinated backyard chickens. Iran J Microbiol. 2018;10(1):65-71.
14.Smialek M, Tykalowski B, Dziewulska D, Stenzel T, Koncicki A. Immunological aspects of the efficiency of protectotype vaccination strategy against chicken infectious bronchitis. BMC Vet Res. 2016;13(1):44.
15. Karimi V, Ghalyanchi Langeroudi A, Hashemzadeh M, Rahimi F, Zabihi Petroudi MT, Farahani RKH, et al., Efficacy of H120 and Ma5 avian infectious bronchitis vaccines in early challenge against QX strain. Virus Dis. 2018;29(1): 1-4.
16. Sarueng E, Wanasawaeng W, Sasipreeyajan J, Chansiripornchai N. Efficacy of live infectious bronchitis vaccine programs against infection by QX-like strain of infectious bronchitis virus. Thai J Vet Med. 2014;44(2):187.
17. Habibi M, Karimi V, Ghalyanchi Langeroudi A, Ghafouri SA, Hashemzadeh M, Farahani RK, et al. Combination of H120 and 1/96 avian infectious bronchitis virus vaccine strains protect chickens against challenge with IS/1494/06 (variant 2)-like infectious bronchitis virus. Acta Virol. 2017;61: 150-160.
18.Terregino C, Toffan A, Beato SM, De Nardi R, Vascellari M, Meini A, et al. Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4/91 serotypes. Avian Pathol. 2008;37(5):487-493.
19. Awad F, Forrester A, Baylis M, Lemiere S, Ganapathy K, Hussien HA, et al. Protection conferred by live infectious bronchitis vaccine viruses against variant Middle East IS/885/00-like and IS/1494/06-like isolates in commercial broiler chicks. Vet Rec Open. 2015;2(2):e000111.
20. Pohuang T, Tanasatian S, Sasipreeyajan J. Efficacy of different vaccination programs of live 4/91 strain against Thai QX-like infectious bronchitis virus in broiler chickens. Thai J Vet Med. 2016; 46(3):419.
21. De Wit J, Detection of infectious bronchitis virus. Avian Pathol. 2000;29(2):71-93.
22. De Wit J, Mekkes DR, Koouwenhoven B, Verheijden JH. Sensitivity and specificity of serological tests for infectious bronchitis virus antibodies in broilers. Avian Pathol. 1997;26(1): 105-118.