Evaluation of a Novel Mutant Strain-Enhanced Inactivated Vaccine for Controlling Infectious Bursal Disease in Chickens

Shuai Yuan*

Washington University in St. Louis, St. Louis, MO 63130, The USA

Abstract: Infectious bursal disease (IBD) is a highly contagious viral infection caused by the Infectious bursal disease virus (IBDV), primarily affecting chickens and turkeys. This disease targets immature lymphocytes, causing immune suppression and lymphoid organ damage. IBD's history dates back to its discovery in 1957, initially named Gumboro disease. Over time, it spread globally, posing significant challenges to the poultry industry. This article presents an overview of IBD, including its epidemiology, clinical symptoms, and vaccination strategies. The study also evaluates the efficacy of a novel mutant strain of IBDV in an inactivated vaccine through antibody titers and bursa index analysis.

1. Introduction

Infectious bursal disease (IBD) is a viral infection caused by IBDV, impacting poultry globally. It affects immature lymphocytes, causing immune suppression and organ damage. First identified as Gumboro disease in 1957, it spread widely, challenging the poultry industry. IBD spreads through respiratory, conjunctival, and digestive routes, with peak virulence at 3-11 days post-infection. Infected birds shed IBDV in feces, contaminating surroundings. Mainly affecting young chickens, it leads to immunosuppression and vulnerability to other infections. Symptoms include disheveled feathers, drooping wings, appetite loss, muscle tremors, and necrosis/atrophy of the bursa. IBD's economic impact drives prevention strategies.[1] Live attenuated and inactivated vaccines are used, each with pros and cons. Attenuated vaccines induce responses but may damage bursa. Inactivated vaccines are less damaging and unaffected by maternal antibodies, but need repeated doses.This study evaluates an inactivated vaccine with a novel IBDV strain. Antibody titers and bursa index will assess its efficacy against bursal lesions. This research enhances IBD prevention strategies, aiding control of this contagious disease.

2. Overview of infectious, bursal disease

Infectious bursal disease (IBD) is an acute, highly contactable, contagious disease caused by infection with Infectious bursal disease virus (IBDV), mainly infecting chickens and turkeys. The disease mainly affects immature and early differentiated lymphocytes, destroying the structure of lymphoid organs and severely impairing their normal immune function; it also causes necrosis of the thymus and spleen as well as atrophy of the bursa, leading to immunosuppression. This disease was discovered in 1957 in a flock of poultry in Gumboro, Delaware (USA), and was therefore called Gumboro disease; in 1962, a study first found that the kidneys of chickens that died from this disease were extremely enlarged, and therefore called "avian nephropathy" ; and later, a study was conducted on chickens isolated from embryos in culture. Afterward, the causative agent of the disease was successfully isolated from infected chickens by chicken embryo isolation and culture, and was officially named as infectious bursal disease by Hitchner in 1970. In China, the disease was first detected in a chicken farm in Beijing in 1979 by Zhou Jiao et al. and IBDV was isolated by antigenic isolation and electron microscopic observation in 1982. Since its emergence, the disease has posed a great threat to the development of the chicken industry, and with the antigenic variation, especially the emergence of super-strong strains and mutant strains, the prevention and control of the disease has become a major problem in the chicken industry, which poses a greater challenge to researchers. The prevention and control of the disease have become a major problem in the chicken industry with the emergence of antigenic mutations, especially the super-strong and mutated strains, posing even greater challenges to researchers, and the greater social and economic damage it brings has been listed as a "major social and economic problem" by the World Organization for Animal Health (WOAH)[2].

To date, many assays have been used for the detection of IBDV, but the existing assays have one or the other drawbacks, such as the operation procedure, the number of steps, and the number of tests. However, the existing methods have some or other shortcomings, such as complicated operation procedures, long reaction times, easy-to-cause false positives, low sensitivity, poor

*Corresponding author: 327udnhh@gmail.com

detection specificity, dependence on expensive equipment and specialized testing methods. However, the existing methods have some disadvantages, such as complicated operation steps, long reaction time, easy to cause false positives, low sensitivity, poor detection specificity, and dependence on expensive equipment and specialized personnel, which cannot meet the efficient detection of IBDV.

2.1. Epidemiology of IBD

Cosgrove first reported a specific infectious disease that damaged chicken bursa in 1962, which was called "IBD". It is also commonly known as Gumboro disease because the first case was found in Gumboro, USA. Since its discovery, IBDV has spread globally, posing a major threat to the poultry industry worldwide. The disease spread throughout much of the United States between 1960 and 1964 and to Europe between 1962 and 1971 [10]. From 1966 to 1974, IBDV was found in the Middle East, southern and western Africa, India, the Far East, and Australia [10]. In 1979, Liu Fuchi, Zhou Jiao, and others investigated chicken farms in the Beijing suburbs and determined that IBD had been introduced to China based on the onset of symptoms, pathologic dissection, histologic observations, and specific serologic reactions in diseased chickens, and successfully IBDV was isolated. IBDV can infect chickens, turkeys, guinea fowl, ducks, and ostriches, but only produces clinical disease in young chickens. Under natural conditions, IBDV can infect chickens from 2 to 15 weeks of age and is most likely to infect chicks from 3 to 6 weeks of age. It is a highly contactable disease, mainly transmitted through the respiratory, conjunctival, and digestive tracts, with peak virulence occurring between 3 and 11 days after infection. IBDV viruses are excreted in the feces of diseased chickens, which then contaminate the feed, water, and the surrounding environment, thereby infecting chickens in the same chicken house. The disease is not seasonal and can occur throughout the year as long as there are susceptible chickens. In addition, IBDV-infected chicken coops usually fail to immunize against Newcastle Disease, Marek's Disease, and Infectious Laryngotracheitis, etc[2]. This is the result of immunosuppression caused by IBDV.

2.2. Clinical symptoms

Sick chickens in the early stage of the onset of feathers fluffy and disheveled, drooping wings, low spirit, loss of appetite, like to drink water, do not like sports, muscle tremors, often crowded together, sleepy, individual chickens appeared to peck the anus phenomenon, and will also contaminate the surrounding feathers. In the late stage of the disease, the body temperature of the sick chickens returned to normal, the appetite is still abolished, excreting yellow, white foamy feces, resulting in severe dehydration. Diseased chickens have a flaccid anus and appear to be trembling, weak, and shy before death, and can die 1-2 d after the appearance of symptoms , and the disease mortality rate can reach more than half. At autopsy, the bursa of Fasciola gigantica was extensively necrotic or atrophic, and a large amount of yellow gelatinous mucus, hemorrhage, and congestion was seen under the plasma membrane, resulting in a "purple grapes-like" appearance; severe hemorrhage in the chest and legs, and the liver was a yellowish color, with a large number of lesions, such as white ice-flake necrosis, around the liver in some diseased chickens[3] . The subclinical type of infection has no significant clinical manifestations, but in some specific antibodies can be detected in certain chickens.

2.3. Classification of avian adenoviruses

According to the latest adenovirus classification published by the International Committee on Classification of Virology in November 2021, adenoviruses are classified into 6 genera and 86 species. According to the latest classification standard published by the International Committee on Virology in November 2021, adenoviruses are categorized into 6 genera and 86 species, and the adenoviruses capable of infecting poultry are listed in Table 1.[4]

Table 1 Adenoviruses Capable of Infecting Poultry According to the Latest Classification by the International Committee on Virology

Genus	Subgroup	Type	Serotypes	Susceptible host
Avian adenovirus genus	I subgroup avian adenovirus	Avian adenovirus A	FAdV-1	Chickens
		Avian adenovirus B	FAdV-5 FAdV-4	
		Avian adenovirus C	FAdV-10 FAdV-2 FAdV-3	
		Avian adenovirus D	FAdV-9 FAdV-11 FAdV-6 FAdV-7	
		Avian adenovirus E	FAdV-8a FAdV-8b	
		Duck adenovirus B	DAdV-2	Duck
		Goose Adenovirus A	GodV-1	Goose
		Pigeon adenovirus	PiAdV-1	Pigeon
		Turkey adenovirus B	TAdV-1 TAdV-2	Turkey
		Falcon	FaAdV-1	Falcon

		adenovirus A		
Sialidase adenovirus genus	Subgroup II avian adenovirus	Turkey adenovirus A	TAdV-3	Turkey
		Raptor adenovirus A	RAdV-1	Eagle, falcon, etc.
High AT Adenovirus genus	Subgroup III avian adenovirus	Duck adenovirus A	DAdV-1(EDSV)	Chicken, duck
Mammalian adenovirus genus	/	Mammalian adenovirus	/	Human, cow, pig, rat, etc..
Fish adenovirus genus	/	Fish adenovirus	/	Beluga
Turtle adenovirus genus	/	Turtle adenovirus	/	Turtles

3. Live attenuated and inactivated vaccines for IBD

Traditional live attenuated and inactivated vaccines are commonly used to prevent IBD. Live vaccines are either artificially induced attenuated lines, or natural attenuated lines with good immunogenicity, or vaccines derived from heterologous weak strains. Live attenuated IBDV vaccines are used to prevent IBDV infection and successfully control the disease when used at the appropriate time of vaccination, and they can replicate in the bursa while inducing both humoral and cellular immunity. Most of these live vaccines may cause atrophy of the bursa during replication, followed by mild or severe lesions due to time depletion of lymphocytes. Generally, live IBDV vaccines used in the poultry industry have been passed on continuously in tissue culture, in order to maintain the immune response elicited by the parental virus, in egg or embryo-derived tissues while attenuating the virulence of the vaccine to cause clinical disease or significant immunosuppression Most conventional live IBDV vaccines are based on classical strains. Most inactivated IBD vaccines are formulated as water-in-oil emulsions, which can usually incorporate a variety of antigens. inactivated IBD vaccines can also induce IBDV-specific T cells and immune responses, and inactivated IBD vaccines must have high antigenic levels to induce immunity to the organism, thereby preventing larvae from being infected by IBDV variants. The advantage of the inactivated vaccine is that it does not damage the bursa, it is not affected by the maternal antibodies, and it does not have any effect on the maternal antibodies, and it is possible to immunize the chickens at the same time, regardless of the maternal antibodies[5]. However, its disadvantage is that it requires repeated immunization, which increases the cost considerably. The inactivated IBDV vaccine is most effectively used in the primary booster program, and the live attenuated IBDV vaccine is used as the primary immunization vaccine.

3.1. Evaluation of the efficacy of a new influenza glandular multiplex inactivated vaccine containing a novel mutant strain of IBDV

In this study, a novel mutant strain of IBDV isolated from chicken embryos was added to the New Flow inactivated glandular vaccine for immunization. A novel mutant strain of IBDV was added to a new flow of inactivated French gland vaccine to immunize chickens for immunization efficacy tests, and the ability of the vaccine to effectively control the current epidemiology of infectious bursal disease was investigated through the detection of antibodies and bursal index.[6]

3.2. Materials

3.2.1. Test animals

Sections 50 chickens of 1-day-old Hyland Brown strain from a certain company were selected as large flock hens and normally immunized; 70 chickens of 1-day-old Hyland Brown strain from a certain company were selected as sentinel chickens, i.e., not immunized with any vaccine. The 70 birds of 1-day-old Hyland Brown strain from a certain company were set up as sentinel chickens, i.e., they were not immunized with any vaccine and kept until 7-10 days of age[7]

3.2.2. Sampling by Dissection

Ten sentinel chickens and five immunized flock chickens were sampled at 12, 26, 42, 54 and 70 days of age respectively. Before sampling, the chickens were weighed and recorded; after that, the chickens were dissected and their blood was collected, about 2 mL of blood was collected from each chicken and put into a sterile test tube. After that, the chickens were dissected and blood was collected, and about 2 mL of blood was collected from each chicken and put into sterile test tubes, and a total of 15 samples were collected each time . The chickens were dissected and observed whether the bursa of the chickens were bleeding, shrinking, and other abnormal symptoms and photos were taken and kept; the bursa was also collected, and the bursa of the same age was combined into one mixed sample.[8]The bursa should be collected and combined with the bursa of the same age to form a mixed sample.

3.3. Bursa index analysis

The bursa of Fasciola was removed and weighed with a precision 0.01 g electronic scale to obtain the weight of the organ, and the same data was also recorded and bursa index was calculated. Bursa index (%) = bursa mass (mg)/living mass (g) × 100. The data were analyzed and processed using the GraphPad Prism 8 software package.[9]

3.4. Results

3.4.1. Antibody test results

Ten sentinel hens and large flock hens were randomly collected at 14, 28, 42, 56 and 70 days of age, respectively, for IBDV antibody test. The results showed that the average antibody titer of sentinel chickens dropped to the lowest at 28 days of age, and then gradually increased, indicating that the sentinel chickens had the lowest antibody titer at 28 days of age. The results showed that the mean antibody titer of sentinel chickens dropped to the lowest at 28 days of age, and then gradually increased, indicating that the sentinel chickens were infected with IBDV; while the mean antibody titer of the large flock chickens gradually increased after immunization. The average antibody titer of the large flock gradually increased after immunization with the vaccine, as shown in Table 2.

Table 2 Bursal antibody titers of sentinel chickens and large flocks of chickens at different days of age

Days of age/day	Mean antibody titer	
	A large group of chicken	The sentry chicken
12	5402	12516
26	11208	1523
42	13356	5203
54	14537	13254
70	14589	15236

3.4.2. Results of bursa index analysis

Ten randomly collected sentinel chickens and five large flock chickens were dissected. 42 days old (Table 2) sentinel chickens showed punctate and diffuse hemorrhages in the bursa. At 42 days of age (Table 2), the bursa of sentinel chickens showed punctate and diffuse hemorrhages and no obvious hemorrhages were seen in large flocks compared to sentinel chickens. At 54 days of age (Table 2), the bursa of sentinel chickens was atrophied, and the bursa of large flock chickens showed no obvious lesions; at 70 days of age (Table 2), the bursa of sentinel chickens was atrophied. at 70 days of age (Table 2), the bursa of sentinel chickens was obviously atrophied, with severe hemorrhage in some parts, and the bursa of large chickens was slightly hemorrhagic, without atrophy. The bursa of sentinel chickens was obviously atrophied at 70 days of age (Table 2). This shows that the vaccine is effective in preventing bursal lesions caused by the new mutant strain of IBDV.

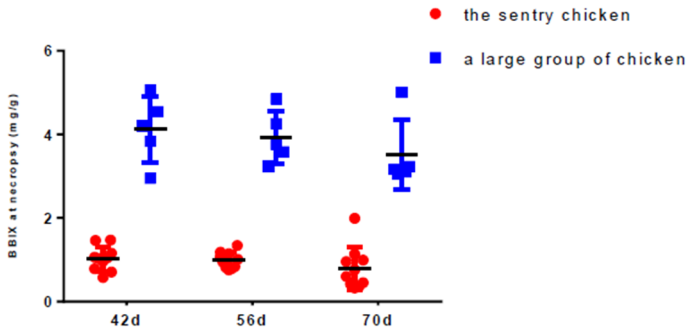

Figure 1 Comparison of bursa index between sentinel chickens and large flocks at different days of age

It was found that the bursal index of normal chickens was 4.10±0.45 at 42 days of age, 3.72±1.06 at 56 days of age, and 3.63±0.11 at 70 days of age. The results of the present experiment showed that the bursal index of sentinel chickens was less than 1, and the bursal index of large flocks of chickens was close to the normal value after immunization, which indicated that the inactivated vaccine prepared in the present study had a certain effect on the bursal atrophy, as shown in Figure 1.

3.4.3. Effect

Among the target organs of IBDV infection, the bursa is the main site of maturation and differentiation of avian B lymphocytes. IBDV can replicate in the bursa and impede the growth of lymphocytes, leading to B cell necrosis and apoptosis, accompanied by B cell exhaustion and deterioration of the bursal tissues [10]. This results in damage to the bursa and impairment of the immune system in chickens, making them less resistant to other infectious diseases and thus reducing immunity to vaccines.

In the present study, it was found that severe atrophy of the bursa was observed at week 6 in never vaccinated sentinel chickens. In immunized sentinel chickens and large flocks, atrophy was observed from week 7 after co-housing. In other infected large flocks, severe atrophy of the bursa was demonstrated and the 56-day-old bursal index was severely lower than the 56-day-old bursal index in normal flocks. The IBD virus (IBDV) isolated from the bursa of unimmunized sentinel chickens was designated as a wild strain of IBDV.

4. Conclusion

Infectious bursal disease (IBD) is a viral infection caused by IBDV, impacting poultry globally. It affects immature lymphocytes, causing immune suppression and organ damage. First identified as Gumboro disease in 1957, it spread widely, challenging the poultry industry. IBD spreads through respiratory, conjunctival, and digestive

routes, with peak virulence at 3-11 days post-infection. Infected birds shed IBDV in feces, contaminating surroundings. Mainly affecting young chickens, it leads to immunosuppression and vulnerability to other infections. Symptoms include disheveled feathers, drooping wings, appetite loss, muscle tremors, and necrosis/atrophy of the bursa. IBD's economic impact drives prevention strategies. Live attenuated and inactivated vaccines are used, each with pros and cons. Attenuated vaccines induce responses but may damage bursa. Inactivated vaccines are less damaging and unaffected by maternal antibodies, but need repeated doses.

This study evaluates an inactivated vaccine with a novel IBDV strain. Antibody titers and bursa index will assess their efficacy against bursal lesions. This research enhances IBD prevention strategies, aiding the control of this contagious disease.

References

1. Orakpoghenor, Ochuko, Sunday B. Oladele, and Paul A. Abdu. "Infectious bursal disease: Transmission, pathogenesis, pathology and control-an overview." World's Poultry Science Journal 76.2 (2020): 292-303.
2. Zhang, Wenying, et al. "The Over-40-years-epidemic of infectious bursal disease virus in China." Viruses 14.10 (2022): 2253.
3. Ray, Susim Mukul, Udi Ashash, and S. Muthukumar. "A field study on the evaluation of day-of-hatch and in grow-out application of live infectious bursal disease virus vaccine in broiler chickens." Poultry science 100.8 (2021): 101252.
4. Trapp, Johanna, and Silke Rautenschlein. "Infectious bursal disease virus' interferences with host immune cells: what do we know?." Avian Pathology 51.4 (2022): 303-316.
5. Eladl, Abdelfattah H., et al. "Immunostimulant effect of a mixed herbal extract on infectious bursal disease virus (IBDV) vaccinated chickens in the context of a co-infection model of avian influenza virus H9N2 and IBDV." Comparative Immunology, Microbiology and Infectious Diseases 72 (2020): 101505.
6. Fitzgerald, Scott D., et al. "Adenovirus infections." Diseases of poultry (2020): 321-363.
7. Harrach, Balázs, et al. "A screening of wild bird samples enhances our knowledge about the biodiversity of avian adenoviruses." Veterinary Research Communications 47.1 (2023): 297-303.
8. Mató, Tamás, András Medveczki, and István Kiss. "Research Note:"Hidden" infectious bursal disease virus infections in Central Europe." Poultry Science 101.8 (2022): 101958.
9. Zhang, Yu, et al. "A novel inactivated bivalent vaccine for chickens against emerging hepatitis-hydropericardium syndrome and infectious bursal disease." Veterinary Microbiology 266 (2022): 109375.
10. Dey, Sohini, et al. "Infectious bursal disease virus in chickens: prevalence, impact, and management strategies." Veterinary Medicine: Research and Reports (2019): 85-97.