Continental Veterinary Journal

Journal homepage: www.cvetj.com

Research Article

Preparation and evaluation of oil adjuvanted inactivated bivalent vaccine against Newcastle disease virus and Infectious Bronchitis virus

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ARTICLE INFO

ARTICLE HISTORY: CVJ-23-1009

Received 17 October 2023
Revised 20 December 2023
Accepted 23 December 2023
Published online 31 December 2023

Keywords:

Paramyxovirus type 1
Gammacoronavirus
Newcastle disease
Infectious bronchitis virus
Vaccine
Poultry birds

ABSTRACT

Newcastle disease and infectious bronchitis disease are the most lethal diseases of poultry currently caused by Newcastle disease virus (NDV) and infectious bronchitis virus (IBV). These viruses belong to Paramyxovirus type 1 and Gammacoronavirus, respectively. Both viruses mainly infect the respiratory and reproductive systems of poultry birds. These infections in poultry lead to a decrease in egg and meat production and cause huge economic losses. Major clinical signs and symptoms of these diseases are following such as dropping wings, depression, coughing, diarrhea, male infertility, and sometimes paralysis. Numerous vaccines are commercially available against these infections such as live vaccines and recombinant vaccines. However, the inactivated vaccines are most effective because they are non-replicating, easy to transport, safe against adverse reactions, and no cold chain equipment is required for storage. This research aims to develop an oil-adjuvanted bivalent inactivated vaccine against NDV and IBV based on field strains; to test the efficiency and immune response of oil adjuvant bivalent vaccine on poultry birds under field conditions. For vaccine preparation, samples were collected from the trachea, kidney, and oviduct of the suspected chickens. Samples were isolated in the allantoic cavity and chorio-allantoic membrane (CAM) of embryonated eggs. Viruses were confirmed by different tests such as HA, HI, and ELISA. For vaccine preparation, these confirmed viruses were cultivated on chicken embryonated eggs. After that, both viruses were inactivated by formalin and bivalent vaccines and prepared by adding an oil adjuvant to increase their efficiency. Vaccine quality was checked by inoculating the prepared vaccine in chickens under normal field conditions. The vaccine prepared conferred protective antibody titers against the disease challenge under controlled conditions.

To Cite This Article: Saira R, A Raheem and A Inayat. 2023. Preparation and evaluation of oil adjuvanted inactivated bivalent vaccine against Newcastle disease virus and Infectious Bronchitis virus Continental Vet J, 3(2):119-125.

Introduction

Poultry is economically very important, as it provides protein-rich products such as meat and eggs. Over a period of 10 years, different product of chicken meat increased by 53%, turkeys' meat 13%, duck meat 67%, goose meat 53%, chicken eggs 39% and other eggs production increased by 27%. In China, the total increase in egg production is 78%. It has been observed that 1/3 of the total meat production belongs to poultry, so this is the reason poultry is significantly very important in producing eggs and meat. Many viral diseases such as avian influenza, infectious bronchitis, and Newcastle disease are life-threatening to poultry.

Many poultry industries are trying to prevent these diseases from poultry because these diseases cause huge economic losses (Scanes 2007).
Respiratory diseases like infectious bronchitis and Newcastle disease are significantly very important diseases because they cause major losses to poultry. In 1930, IBV was first identified in the USA and in the UK. It was discovered in 1948. Massachusetts serotype was considered the only serotype for this disease for many years. But in the 1970s and '80s new serotypes were identified, that transferred from infected birds to others and after that IBV spread worldwide (Jones 2010). It is caused by the Gammacoronavirus infectious bronchitis virus (IBV) (OIE 2013). In 1926, NDV was first time reported as a lethal disease. From 2006 to 2009, NDV was considered the 4th most deadly disease. It infects 236 species of birds, which includes chickens, pigeons, turkeys, ducks etc. The infection is caused by Avian Paramyxovirus serotype-1 (AMPV-1) (Kapczynski et al. 2013). NDV have 21 different serotypes designated as APMV-1 to APMV-21 (Anonymous 2013).
Clinical signs are not specific for these diseases but some includes anorexia, hyperthermia and open mouth breathing (Kapczynski et al. 2013). NDV also affects the nervous, gastrointestinal, and respiratory system. In NDV, chickens are unable to produce eggs because NDV severely affect the reproductive system (Dimitrov et al. 2017). Greenish watery diarrhea, depression, drooping wings and in severe condition complete paralysis have been reported. IB affects the upper respiratory tract, female reproductive tract, and epithelial cells in kidney. Moreover, this disease also causes male infertility. Most important clinical signs and symptoms of IBV are following: coughing, ocular and nasal discharge and head shaking along these, losses of egg production, thin, soft, rough egg shell and sometimes elongated eggs are also included (Hassan et al. 2022). To control these diseases; diet, water supply and hygiene conditions must be improved along with the proper vaccination of birds.
The route of transmission of NDV and IBV is almost same as mostly airborne and droplets. Newcastle disease transmitted through fine aerosols, contaminated feed, water, direct contact with suspected animals and sometimes transmission through people have also been identified. Transmission of these infections depends upon the environmental factors such as density, temperature, humidity, host behaviour, host defense mechanism, host age, virus infectivity and strain of virus. Non-living objects are known to be involved in viral transmission. Field workers and equipment are also the source of disease transmission. Direct and indirect transmission can be involved at the same time (Li et al. 2009).

Serological diagnosis of these viruses includes several tests, for example heamagglutination test, heamagglutination inhibition test and enzyme-linked immunosorbent assay. Agar gel precipitation tests can also be performed. These all come under conventional methods. Recently, advanced genetic techniques used for the diagnosis of these viruses are sequencing and real-time reverse transcriptase PCR. These tests confirm the variants of viruses at the genetic level (Anonymous 2013).
Chicken and Poultry play a significant role in our economy, but most lethal viral diseases affect them. So, it is very important to protect poultry from these deadly viruses through vaccination. Both NDV and IBV are undergoing rapid mutation that leads to the development of new viral strains. Due to this, no vaccine has completely eradicated these diseases. So, preparation of vaccine against these diseases is very challenging. Vaccination is very common for the protection against many pathogenic and viral diseases. Some effective vaccines are available commercially for the prevention of outbreaks of these diseases in poultry. Different vaccines such as recombinant vaccines, live attenuated vaccines and adjuvanted vaccines against the ND and IB are available. All of them are very expensive and time consuming and somehow, the outbreak of such disease cannot overcome by using those vaccines (Winterfield 1984). Most commonly, oil adjuvant inactivated vaccine can be used against these viruses. Oil adjuvant can increase the efficiency of inactivated vaccine. Different oil adjuvants can be used in different vaccine such as alum, ssRNA, dsRNA, CpG and combination adjuvants etc. Oil adjuvants increase the immunogenicity and retaining power of vaccine. The aim of this research is to design bivalent oil adjuvant inactivated vaccine because this vaccine can kill these two viruses (ND and IB) at the same time moreover preparation of two separate vaccines against these viruses takes a lot of time and expensive. This bivalent vaccine was tested in chickens to confirm its effectiveness and stability (McKee et al. 2007).

Materials and Methods

Sample collection

Organ samples for the Infectious bronchitis virus and Newcastle disease virus isolation were collected separately from infected birds and dead birds. Different infected organs such as trachea, kidney, oviduct, lungs, liver, and spleen were collected into sterile plastic bags. Samples were kept on ice or frozen as quickly as feasible. Samples of both viruses were put in cold transport media (Phosphate Buffer Saline) with penicillin (10,000 [IU]/ml) and streptomycin (10 mg/ml). Sample were placed at 4°C for short term storage and at -80°C for long term storage (OIE 2013).

Isolation of viruses

Samples were chopped into tissues and then grinded by using sterile mortar and pestle for isolation of viruses. Tissue suspensions (10–20% w/v) were prepared in sterile phosphate buffered saline (PBS) for inoculation of both viruses. For chicken tracheal organ culture (TOC) inoculation, tissue culture medium was used only in IBV (Cook et al. 1976). Tissue suspensions are cleared by low-speed centrifugation at 1000g and at 25 °C temperature for 10 minutes separately for both viruses. Supernatant were collected and filtered via bacteriological filters (0.2). Embryonated eggs were used for viral isolation. These eggs were procured from SPF hens, which were not exposed to IBV through infection or vaccination. Before the inoculation process, candling was performed to mark the location of the air sac in eggs. Holes on eggs were made with egg punch. 0.1–0.2 ml of sample supernatant was inoculated by using a 1ml syringe with tuberculin needle into an allantoic cavity of 9–11-day-old embryos. After inoculation, holes were closed with paraffin wax. Inoculated eggs were incubated at 37°C for 2-7 days. For seven days, eggs were candled every day to check the viability or death of the embryo. Death of the embryo occurring within the first 24 hours were regarded as nonspecific. Allantoic fluids were stored in sterile screw-capped at -20°C for short- term storage and at -60°C for long-term storage separately for both viruses (Anonymous 2013; Spackman 2020).

Identification and detection of viruses:

Different tests were performed for the confirmation of both viruses. Hemagglutination Inhibition (HI), Hemagglutination Assay (HA) (Dimitrov et al. 20017) were performed for NDV detection. Enzyme-linked immunosorbent assay (ELISA) were used for detection of IBV (OIE 2013).

Inactivation of viruses and vaccine preparation:

Old, cultivated viruses (NDV and IBV) were inactivated by formalin. 1% Formalin were mixed with 10ml viral suspension separately for both viruses. These viral suspensions containing formalin were incubated at 37°C for 48 hours. For confirmation of inactivation of both viruses, it was inoculated into separate 9-10 days old embryonated eggs. These eggs were incubated for 3 days at 37°C. After 3 days of incubation, embryonated eggs were observed. Allantoic fluids of dead embryos were collected and tested by Heamagglutination assay for the presence of virus antigen (Pawar et al. 2015). After that, inactivated bivalent vaccines was prepared by mixing both inactivated viruses and Montanide ISA-026 oil adjuvant on a magnetic stirrer (Gough et al. 1977).

Vaccine Efficacy

Vaccine efficiencies were confirmed by inoculating this vaccine in healthy birds. Viability was checked for up to 10 days using conventional methods. The Chickens were divided into 3 groups. One group were injected with commercial vaccine. 2nd group were negative control (non-vaccinated). 3rd group of chickens were injected with prepared bivalent vaccine. After 14 days of vaccination, blood of chickens was collected and antibody titer were checked (Shakal et al. 2013).

Statistical analysis

Statistical analysis was analyzed by analysis of variance (ANOVA) after obtaining the data from field.

Results

The present study was conducted on the preparation and evaluation of oil adjuvanted inactivated bivalent vaccine against Newcastle disease virus (NDV) and Infectious bronchitis virus (IBV). Water in oil (W/O) bivalent vaccine was prepared by using inactivated antigens of NDV and IBV along with Montanide ISA 206 VG (oil adjuvant). Vaccine was prepared using an aqueous to oil ratio of 3:7. Physical compositions of vaccines like sterility, stability and viscosity emulsion type were noted. Vaccine efficacy was assessed based on humoral immune response. The data was statistically analyzed through analysis of variance (ANOVA) by applying the complete randomized design (CRD).

Physical properties of vaccines:

The appearance of experimentally prepared bivalent vaccine was fluidic and milky white. Vaccine was moderately fluidic because Montanide ISA 206 VG promotes less viscosity so that the vaccine easily diffuses i.e. 4 seconds. Vaccine was found to be stable more than 5 weeks at room temperature. Vaccine was observed to be sterile as there was lack of any bacterial or fungal growth on Nutrient agar, MacConkey agar and Sabaroud agar. Experimental vaccine was found to be safe when inoculated in adult broiler chickens, as no local and systemic reaction was observed in any bird up to two weeks post-inoculation.

Table 1: Intra-cerebral Pathogenicity Index (ICPI) of NDV

Clinical Signs	Days								Total Score	
	1	2	3	4	5	6	7	8		
Normal	10	10	10	6	4	0	0	0	40 × 0	= 0
Sick	0	0	0	4	6	8	6	0	24 × 1	= 24
Dead	0	0	0	0	0	2	4	10	16 × 2	= 32
Total	-	-	-	-	-	-	-	-	-	56

Intra-cerebral pathogenicity index (ICPI) of NDV

Intra-cerebral Pathogenicity Index (ICPI) was used to identify the specific pathotypes of Newcastle disease virus (NDV). In this test day old 10 chicks were injected intra-cerebrally with NDV. The

mortality and morbidity of these chicks were observed till 8 days. After 8 days observation, ICPI value was calculated by using appropriate formula. The calculated value was compared with the standard data of values for ICPI which confirmed the Lentogenic strain of NDV.
Mean score per bird per observation = Total Score/ Number of Chickens × Number of Observations = 56/10 × 8 = 0.7
ICPI of harvested virus (NDV) was calculated as 0.7, which indicated that the harvested strain was Lentogenic.

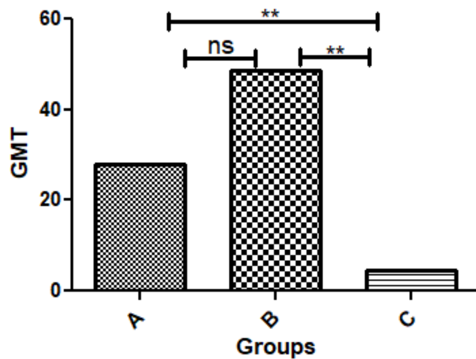

Fig. 1: GMTs of NDV on 7th day of vaccination (21 day of age)

Table 2: GMT of NDV at 7th day of Vaccination (21 days of age)

Samples	Group1		Group 2		Group 3	
	HI	Log_2 HI	HI	Log_2 HI	HI	Log_2 HI
1	64	6	32	5	8	3
2	32	5	64	6	4	2
3	16	4	64	6	4	2
4	32	5	64	6	4	2
5	16	4	32	5	4	2
GMT	**27.8**		**48.5**		**6.0**	

Humoral Immune Response
Birds that had received experimental vaccinations had their serum samples taken up to two weeks after the regular interval of one-week post-immunization (14th day). Collected serum samples were subjected to haemagglutination inhibition (HI) assay to measure the antibody titre against NDV and IBV.

Log_2 HI titre against NDV at 7th day of vaccination (21 days of age)
At 7th day of vaccination, the antibody titres against NDV of randomly selected 5 birds from each of the three groups ranging from 2 to 6. GMTs of group A, B and C were recorded as 27.8, 48.5 and 6.0, respectively (Table 2; Fig. 1). Statistical difference in the GMTs of group A and B was recorded as non-significant whereas, vaccinated groups (A and B) had significant difference as compared to non-vaccinated (group C).

Log_2 HI titre against NDV at 14th day of vaccination (28 days of age)
At 14th day of vaccination, the antibody titres against NDV of randomly selected 5 birds from each of the three groups ranging from 2 to 7. GMTs of group A, B and C were recorded as 36.7, 84.4 and 5.2 respectively (Table 3; Fig. 2). Statistical difference in the GMTs of group A and B was recorded as significant whereas, vaccinated groups also showed significant difference as compared to non-vaccinated group C.

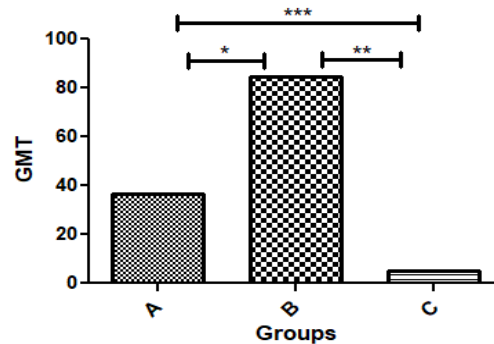

Fig. 2: GMTs of NDV at 14th day of vaccination (28 day of age)

Table 3: GMT of NDV at 14th day of Vaccination (21 days of age)

Samples	Group1		Group 2		Group 3	
	HI	Log_2 HI	HI	Log_2 HI	HI	Log_2 HI
1	64	6	128	7	4	2
2	32	5	64	6	8	3
3	32	5	128	7	8	3
4	32	5	64	6	4	2
5	32	5	64	6	4	2
GMT	**36.7**		**84.4**		**5.2**	

Log_2 HI titer against IBV at 7th day of vaccination (21 days of age)

At 7th day of vaccination, the antibody titres against IBV of randomly selected 5 birds from each of the three groups ranging from 2 to 7. GMTs of group A, B and C were recorded as 40.8, 97.0 and 5.2 respectively (Table 4; Fig. 3). Statistical difference in the GMTs of group A and B was recorded as significant whereas, vaccinated showed significant difference as compared to non-vaccinated.

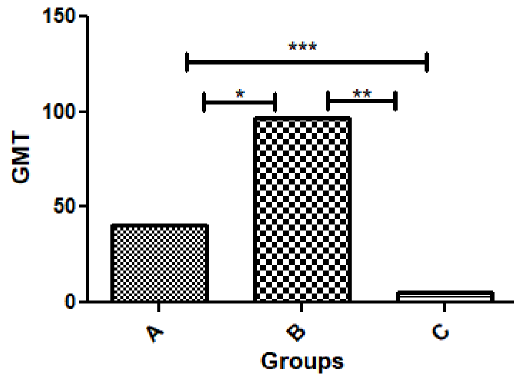

Fig. 3: GMTs of IBV on 7th day of vaccination (21 day of age)

Table 4: GMT of IBV at 7th day of Vaccination (21 days of age)

Samples	**Group1**		**Group 2**		**Group 3**	
	HI	**Log2 HI**	**HI**	**HI**	**Log2 HI**	**HI**
1	32	5	64	6	4	2
2	32	5	128	7	8	3
3	64	6	64	6	8	3
4	54	6	128	7	4	2
5	32	5	128	7	4	2
GMT	**40.8**		**97.0**		**5.2**	

Log2 HI titer against IBV at 14th day of vaccination (28 days of age)

At 14th day of vaccination, the antibody titres against IBV of randomly selected 5 birds from each of the three groups ranging from 2 to 7. GMTs of group A, B and C were recorded as 55.7, 111.4 and 5.2 respectively (Table 5; Fig. 4). Statistical difference in the GMTs of group A and B was recorded as significant whereas, vaccinated group showed significant difference as compared to non-vaccinated.

Discussion

In many nations, the poultry industry has grown to be a significant economic sector. The poultry industry is the largest livestock group in which more over 30% of all animal protein comes from poultry. However, only 20% of all poultry are raised for commercial, which is the main basis for this production. Poultry is the world's largest animal stock, which is measured by the number of animals. In terms of animal production, poultry is a significant segment that is dominated by backyard flocks, particularly in developing nations. In these countries, villagers raise chicken as a source of additional income and to meet the dietary needs of their families. Major illnesses in poultry include low biosecurity measures and a higher risk of infectious diseases, including infectious bronchitis and Newcastle disease. (Abdisa and Tagesu 2017).

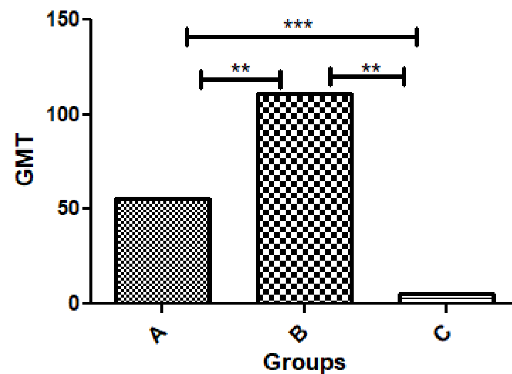

Fig. 4: GMTs of IBV at 21st day of vaccination (28 day of age)

Table 5: GMT of IBV at 14th day of Vaccination (28 days of age)

Samples	**Group1**		**Group 2**		**Group 3**	
	HI	**Log2 HI**	**HI**	**HI**	**Log2 HI**	**HI**
1	32	5	128	7	4	2
2	64	6	128	7	8	3
3	64	6	64	6	8	3
4	64	6	128	7	4	2
5	64	6	128	7	4	2
GMT	**55.7**		**111.4**		**5.2**	

Disease-related issues and deterioration of the environment frequently occur in large-scale rearing facilities where chickens are subjected to stressful conditions and cause severe economic losses. The usage of veterinary pharmaceuticals has significantly increased during the past few decades as a result of disease prevention and

control measures (Kabir 2009). Vaccination is the main step to overcome these lethal diseases.
The present study is about the preparation of oil adjuvanted inactivated bivalent vaccine against Newcastle disease virus and infectious bronchitis virus. The aim of this research is to test the efficiency and immunological response of oil adjuvant bivalent vaccine on poultry birds under field condition. Vaccine quality was checked by inoculating the prepared vaccine for 3 groups chickens (each group containing 20 chickens) under normal field conditions. For experimental design, 14 days old broilers chickens were used. 1st dose of prepared vaccine was injected at 0th day when chickens were 14th day age. 2nd dose of prepared vaccine was injected at 7th day when chickens were 21st day age. Five randomly chosen birds had their blood samples taken at the age of 21 and 28, at 7th and 14th day of vaccination, respectively. 28th day post vaccination, chickens were challenged with antigen and were monitored for morbidity and mortality.

In my study three groups of birds were examined by treated with commercial vaccine, prepared bivalent and one kept as a control. Fathy et al. (2019) compared the field efficacy of a freshly made inactivated classical and variation 2 vaccine with commercial (ND and IB) in layer chickens. Comparing the immunized chickens from both groups to the unvaccinated control groups, they were assessed for resistance to heterologous and homologous challenge. In this experiment, four estimated variables were used: egg production percentage, ELISA humoral immunity after vaccination and challenge, histopathological findings from tracheal and oviduct samples after challenge and real-time RT-PCR challenge strain detection. The outcomes showed that the produced vaccine gave superior egg production after challenge and stronger protective antibody titers in serum than the commercial inactivated (NDV & IBV) one. Regarding its impact on the oviduct, the identification of IB viruses shedding was restricted to the trachea, except in control groups. The current investigation shows that locally produced bivalent IBV vaccination is efficacious in commercial layers; moreover, combining inactivated variant and traditional IBV strains offers extensive protection in places where the virus is endemic against both homologous and heterologous challenge. The results of both research are similar as my research is on inactivated bivalent vaccine against NDV and IBV, which showed 80% affectivity and it also enhance the immune response of birds just like the above research which showed high antibody response.
In this study, bivalent vaccine against Newcastle disease virus and infectious bronchitis virus was prepared which showed 80% affectivity after field trials. The similar study was conducted by Tan et al. (2020) who developed the recombinant thermostable Newcastle disease virus vaccine which express multiple epitopes cassette of infectious bronchitious disease virus against IBV and NDV. Many live attenuated and inactivated vaccines are commercially available. Most killed inactivated and live attenuated vaccines are used. However, NDV and IBV live vaccine cause low immunity. As live vaccines are sensitive to cold temperature. So, for long term storage of live vaccines, refrigerators, and cold chain equipment (CCE) are very necessary to maintain the quality, effectiveness, and immunity of vaccines. So, preparations for these types of vaccines are very costly and preparation for recombinant vaccine is very challenging. He found that some avirulent NDV strains do not require cold conditions because they are naturally thermostable. He designed rLS-T-HN-T/B recombinant thermostable vaccine under optimum conditions. The infectivity titer was tested between rLS-T-HN-T/B strain and LaSota strain at the same temperature 56c from 5 to 15 minutes. Then test indicated that rLS-T-HN-T/B showed more protection against NDV and IBV and match the criteria of thermostability than parental LaSota vaccine. This indicates that addition of IBV multiple epitopes cassette in rLS-T-HN-T/B strain did not affect thermostability of vaccine. After trail on infected chicken the rLS-T-HN-T/B showed 100% and 90% affectivity against NDV and IBV respectively. And LaSota vaccine showed 20% protection against NDV and IBV. This study indicates that bivalent recombinant vaccine protect poultry from both lethal diseases NDV as well as IBV under low cost and without the cold chain equipment. My study also less expensive as compared to above research on recombinant thermostable vaccine against virus.
Abodalal (2017) prepared adjuvanted live vaccine against infectious bronchitis and evaluated its efficiency by using Montanide gel 01 adjuvant. It was discovered that augmenting the IB live vaccination with Montanide gel 01 produced vaccine of a higher efficiency when applied as spray as well as intranasally. The IB- Montanide gel 01 produced better levels of humoral and cellular immunity than the traditional, non-adjuvanted IB vaccinations. Additionally, when exposed to the virulent IB strains, the gel 01 IB vaccine demonstrated greater protection than the conventional vaccines. Additionally, it was shown that the gel IB vaccination could employed as a spray as an intranasal vaccine delivery method, particularly for mass vaccination in the enormous chicken. The outcomes demonstrated that using the Montanide gel 01 vaccination boosted the IB vaccine's safety margin and improved its efficacy. My study indicated somewhat better results by using Montanide ISA 206 in the bivalent vaccine against Newcastle disease and infectious bronchitis. Firstly, above study is monovalent

vaccine against one virus infectious bronchitis and my prepared bivalent vaccine produce immunity against two viruses IB and ND. Above study used Montanide gel 01 adjuvant for live vaccine and my study Montanide ISA 206 for inactivated vaccine and both gives similar results. These adjuvants increased the efficacy of vaccine as it can retain itself and slowly release the vaccine into body, which produce long term immunity.

In a study conducted by Li et al. (2012), the researchers observed the effects of Sargassum pallidum polysaccharides (SPP) on immunological responses in chickens. They analyzed the antibody titers and lymphocyte proliferation in hens after administering SPP as an adjuvant in Newcastle disease (ND), infectious bronchitis (IB), and avian influenza (AI) vaccines. The combination vaccines containing SPP were given at concentrations of 10, 30, and 50 mg/mL, while an oil adjuvant vaccination served as the control. Results showed that at 30 mg/mL SPP concentration, the ND, IB, and AI antibody titers, as well as lymphocyte proliferation, increased. In the present research, Montanide oil adjuvant was used instead of Sargassum pallidum polysaccharides (SPP) because it has a less fluidic nature and is more easily tolerated. Nonetheless, the study yielded similar results, indicating that an appropriate dose of SPP could serve as a safe and effective immune stimulator option to achieve early and durable prophylaxis in vaccinations.

Conclusion

The study developed and evaluated an oil-adjuvanted inactivated bivalent vaccine targeting infectious bronchitis virus (IBV) and Newcastle disease virus (NDV). The vaccine prepared conferred protective antibody titers against the disease challenge under the controlled conditions.

Funding

This current study was conducted without any external funding.

Acknowledgement

All the authors acknowledge technical support of Institute of Microbiology, University of Agriculture, Faisalabad.

Competing Interest

The authors declare that they have no relevant financial or non-financial interests to disclose.

Consent for publication

All the authors consented to publication.

Data Availability

Tye data can be demanded from corresponding author on a reasonable request.

Author Contribution

AR and AI designed the study SR conducted experiment and wrote the paper. AR and AI revised the manuscript.

References

Abdisa T and Tagesu T, 2017. Review on Newcastle disease of poultry and its public health importance. Journal of Veterinary Science and Technology 8:1000441.

Dimitrov KM, Afonso CL, Yu Q and Miller PJ, 2017. Newcastle disease vaccines—A solved problem or a continuous challenge? Veterinary Microbiology 206:126-136.

Gough RE, Allan WH and Nedelciu D, 1977. Immune response to monovalent and bivalent newcastle disease and infectious bronchitis inactivated vaccines. Avian Pathology 6:131–142.

Hassan MSH, Buharideen SM, Ali A, et al. 2022. Efficacy of Commercial Infectious Bronchitis Vaccines against Canadian Delmarva (DMV/1639) Infectious Bronchitis Virus Infection in Layers. Vaccines (Basel) 10:1194.

Jones RC, 2010. Europe: History, current situation and control measures for infectious bronchitis. Brazilian Journal of Poultry Science 12:125-128.

Kabir SML, 2009. The role of probiotics in the poultry industry. International Journal of Molecular Sciences 10:3531-3546.

Kapczynski DR, Afonso CL and Miller PJ, 2013. Immune responses of poultry to Newcastle disease virus. Developmental and Comparative Immunology 41:447-453.

Li X, Chai T, Wang Z, Song C, Cao H, Liu J, Zhang X, Wang W, Yao M and Miao Z, 2009. Occurrence and transmission of Newcastle disease virus aerosol originating frOccurrence,d chickens under experimental conditions. Veterinary Microbiology. 136(3-4): 226-32.

McKee AS, Munks MW and Marrack P, 2007. How Do Adjuvants Work? Important Considerations for New Generation Adjuvants. Immunity 27:687-690.

OIE, 2013. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organization for Animal Health 9:1185-1191.

Pawar SD, Murtadak VB, Kale SD, Shinde PV and Parkhi SS, 2015. Evaluation of different inactivation methods for high and low pathogenic avian influenza viruses in egg-fluids for antigen preparation. Journal of Virological Methods 222:28-33.

Scanes CG, 2007. The global need for poultry science education, research, and outreach. Poultry Science 86:1285-1286.

Winterfield RW, 1984. Vaccination of chickens with Newcastle disease and infectious bronchitis vaccines administered singly and in combination. Poultry Science 63:182-184.