

Immunogenicity and Efficacy Evaluation of Vero Cell-Adapted Infectious Bursal Disease Virus LC-75 Vaccine Strain in Ethiopia

Wakjira Kebebe

National Veterinary Institute

Molalegne Bitew

Ethiopian Biotechnology Institute

Fufa Dawo

College of Veterinary Medicine and Agriculture

Bedaso Mammo

College of Veterinary Medicine and Agriculture

Hawa Mohammed

National Veterinary Institute

Martha Yami

National Veterinary Institute

Belayneh Getachew

National Veterinary Institute

Takele Abayneh

National Veterinary Institute

Esayas Gelaye (✉ esayasgelaye@gmail.com)

National Veterinary Institute <https://orcid.org/0000-0002-7329-8632>

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Abstract

Background: Infectious bursal disease virus (IBDV) is an avian viral pathogen that causes infectious bursal disease (IBD) of chickens. The disease is endemic in Ethiopia since 2002 and vaccination is the major means of disease prevention and control. IBD vaccine is produced in Ethiopia using primary chicken embryo fibroblast (CEF) cell; which is time-consuming, laborious, and uneconomical. The present study was carried out to develop cell-based IBDV LC-75 vaccine using Vero cells, and to evaluate the immunogenicity and protection level.

Results: Identity of the vaccine seed was confirmed using gene-specific primers using reverse transcription polymerase chain reaction. Confluent monolayer of Vero cells was infected with vaccine virus and serial passage continued till passage ten. Characteristic virus induced cytopathic effect was observed starting from passage 2 on the third day post-infection. The infectious titer of adapted virus showed a linear increment along the passage level. Virus induced specific antibody was determined using indirect ELISA after vaccination of 14 days old chicks through ocular route. Accordingly, the antibody titer measured from Vero cells vaccinated chicks revealed similar level with the currently available CEF cell-based vaccine. Chicks vaccinated with Vero cell adapted virus showed complete protection against very virulent IBDV, while unvaccinated group had 60% morbidity and 25% mortality.

Conclusions: The IBDV vaccine strain well adapted on Vero cells and found to be immunogenic induces antibodies development and successfully protects chicks against challenging with the circulating field IBDV isolate. Hence, it is recommended to produce IBD vaccine using Vero cell culture with enough quantity to conquer the limitations using CEF cells and thus to vaccinate chicks to protect against IBDV infection.

Background

Infectious bursal disease (IBD also known as Gumboro) is an immunosuppressive disease of chicken and is the second priority chicken disease in Ethiopia [1] that needs to be controlled primarily through vaccination. The etiological agent is IBD virus (IBDV) belongs to the family *Birnaviridae* which is a non-enveloped, icosahedral in shape with double-stranded and bi-segmented ribonucleic acid (RNA) genome [2]. The majority of chicken production in Ethiopia is under small scale backyard management system and they serve as source of egg and meat consumptions and for generating cash income. In general, Ethiopia has around 60.5 million poultry [3]. Losses due to chicken mortality occur in different age groups due to presence of different poultry diseases including IBD. Among the causes of the poultry disease in Ethiopia, IBD is one of the diseases which is challenging chicken production and product quality. IBD was first reported in Ethiopia in 2002 at privately owned commercial poultry farm in which 45-50% mortality rate was reported [4]. Subsequently, IBD has become a second priority problem next to Newcastle disease in both commercial and backyard poultry production systems in Ethiopia despite regular vaccination practices (in commercial farms) using IBDV LC-75 vaccine and improved biosecurity measures [5]. Since then, the circulation of very virulent [6, 7] and/or classical virulent IBD viruses [6] were reported from

different parts of Ethiopia. Vaccination is the cheapest, most practical and economical method of disease control globally. Most control strategies designed in the country do not take into consideration the local chickens which contribute to 99% of the chicken population. Despite that improved chicken genotypes are being distributed to smallholder farmers in the country by different organizations as a means to improve livelihood of the farmers and these development activities failed because of presence of priority endemic poultry diseases like IBD and Newcastle disease. Previously, Tadelle and Ogle [8] reported that chicken vaccination against prevailing diseases in Ethiopia did not take into account local chicken population found under smallholder farmers' management conditions. Starting from 2004, highly pathogenic IBDV strains emerged via mutation and genetic re-assortment [9]. IBD is a contagious and economically important poultry disease in Ethiopia [10]. Unless the chickens get vaccine during their early life (2 to 6 weeks of age), it leads to considerable economic loss. Therefore, vaccination is considered as an important means of protecting birds during their first weeks of life [11]. One of the strategies to prevent and control IBD is to hyper-immunize breeders with vaccines [12]. Although passive immunity promotes good protection of chickens during the first weeks of life, permanent protection against IBD requires the administration of live vaccines [13]. The live IBD vaccines so far developed and in use are categorized as "mild", "intermediate" and "hot" according to their degree of virulence [14]. Mild vaccines are safe for specific pathogen-free (SPF) chickens but are not very effective in the presence of high levels of maternal antibodies or against very virulent strains of IBDV. Hence, the intermediate and hot vaccines are much more effective but may induce moderate to severe lesions in the Bursa of Fabricius [14]. Currently, Ethiopia is producing live intermediate plus IBDV vaccine using LC-75 strain using chicken embryo fibroblast cell (CEF) [7] at the National Veterinary Institute (NVI). Production of IBD vaccine based on CEF cells needs high human labor for preparation of primary cells, costly since it needs use of specific pathogen-free eggs and is time-consuming with a minimum production capacity. Different cell lines such as Vero cells are also suitable for the growth of IBDV so that for vaccine production it is easily manageable and reproducible with a minimum cost but needs proper adaptation of the vaccine strain to the Vero cells. Therefore, in this paper we reported adaptation of IBDV vaccine strain (intermediate plus LC-75) on Vero cell, and evaluation of the immunogenicity and efficacy of Vero cell adapted vaccine strain in target host.

Results

Adaptation of IBDV LC-75 on Vero Cells

The result of the present study showed that the LC-75 strain of IBDV vaccine strain successfully adapted in Vero cell culture starting from passage two to ten. At the first passage, the infected cells remained intact on the surface of tissue culture flask and did not show any CPEs formation. During second passage, minor CPEs were observed starting from day four post-inoculation. However; starting from passage three up to passage ten, visible CPEs happened at day three post-infection by observing typical aggregation, rounding and clumping of large number of cells and detachment of infected cells with few cells floating in media as visualized by inverted microscope (Fig. 1).

Titration of IBDV

The infectivity of adapted IBDV LC-75 to Vero cells was determined by calculating 50% endpoint as described by Spearman Karber method [16]. The total infectious virus \log_{10} TCID₅₀/ml titer of Vero cell adapted passage 2, 3, 5, 7, 9 and 10 were 5.4, 5.60, 5.9, 6 and 6.2, respectively (Fig. 2). The best-fit equation for the log titer increment was $Y = 5.0695 + 0.1104x$ ("x" stands for passage number).

Molecular detection and sequencing

The amplified PCR products were visualized on agarose gel electrophoresis that resulted in the generation of 645 bp expected size amplicon of the VP2 gene (Fig. 3). PCR amplicons obtained from virus-infected Vero cell homogenates were confirmed as IBDV (Fig. 3). The VP2 gene nucleotide sequences of the present LC75 vaccine strain viruses isolated from the different culture passages were deposited in the GenBank with accession numbers of IBDV/LC-75/passage 1 (MK798159), IBDV/LC-75/passage 5 (MK798160) and IBDV/LC-75/passage 10 (MK798161).

Immunogenicity of the developed vaccine

The indirect ELISA test result showed that all chicks contained fewer amount of maternally derived antibody that is within the negative range of the S/P ratio. Seven days post-inoculation of the antigen, the mean S/P ratio for Group 1 inoculated from passage 5 was 0.046 (antibody titer 141.86), for Group 2 inoculated from passage 10 was 0.068 (antibody titer 207.27) and for Group 3 inoculated with CEF cell-based vaccine was 0.094 (antibody titer 283.75). At day 14 post-inoculation S/P ratio was 1.28 (antibody titer 3572.68) for passage 5, 1.53 (antibody titer 4247.67) for passage 10 and 1.76 (antibody titer 4865.72) for CEF based vaccine (Gum 01/18). At day 21 post infections the S/P ratio or antibody titer seems equal with that of day 14 post-inoculation (Fig. 4). The chickens were boosted at day fourteen post-inoculation and, after 7 - 14 day of boosting, the S/P ratio was strongly increased from 1.53 (antibody titer 4247.67) to 2.15 (antibody titer 6307.77) (Fig. 4).

Immunogenicity and efficacy evaluation

The comparison of IBD virus antibodies produced against Vero cell adapted virus from passages 5 and 10 with that of CEF cell based NVI vaccine (Batch Gum 01/18) showed the absence of significant difference between the two IBD vaccines used.

Chick groups vaccinated with Vero cell adapted LC-75 IBDV strain from passages 5 and 10 and from CEF cell based vaccine (Batch Gum 01/18) was found to be safe as no clinical signs and death were observed as compared to the unvaccinated control group (Fig. 5). Starting from day 4 post-inoculations of challenge virus, 60% of chicks from control (unvaccinated) group showed clinical signs such as ruffled

feather, inappetence, difficult movement, yellowish diarrhea and dehydration, while 25% of the unvaccinated chickens died starting from day 6 post-challenge.

Discussion

The CEF cell-based vaccine production needs SPF chicken eggs, nevertheless obtaining of SPF eggs and processing of the embryo needs hard currency to import the SPF eggs and also incurs additional labor cost as its processing is labor-intensive and cannot be easily scaled up for large scale vaccine production. The use of Vero cell line for the IBDV vaccine production could be the best alternative.

In the present study, IBDV LC-75 vaccine strain was successfully adapted in Vero cells and the titer was compared with the IBD vaccine produced using chicken embryo fibroblast cells using the same vaccine strain. The Vero cell adapted IBDV LC-75 vaccine strain at different passages were proved to be immunogenic and protected chickens against challenge with vvIBDV field strain (MB/263/17). Hence, the Vero cell based IBDV vaccine is recommended for ultimate vaccine production and use in Ethiopia. Previously, it was suggested that the use of Vero cells in growing avian viruses could become an economical, less laborious, and continuous and efficient tool with an advantage of measuring virus effects outside the host animal [21] as compared to the laborious preparation of primary CEF cell. We have tested that confluent monolayer of Vero cells, following 36 hours of sub-culturing in growth medium, infected with IBDV LC-75 remained fully intact in passage 1 (P1) up to six days post-infection. When the first passage virus was blindly harvested and passaged (second passage or P2), some changes in Vero cell monolayer began to develop on fourth day of incubation. Monolayer showed rounding of infected cells but complete CPEs of IBDV on Vero cells were not found in this passage too. CPE was observed starting from day three post-infection after the virus became adapted to the cell. This indicated that the virus had adjusted itself to Vero cell culture environment and started replication with good and observable CPEs. Hussain and Rasool [15] also reported that the passage level and the time at which the CPE observed and completed was similar to the present study. On the other hand, Isabela et al. [22] reported that detection of IBD virus titer inoculated on Vero cell was not observed and this could be related to the strain of the IBD virus or passage level used. The current result also indicated presence of difference in time of CPEs observation with that of Silva et al. [21] that reported IBDV CPEs in Vero cells at 18 hours post-infection. The titer of Vero cell adapted IBDV LC-75 strain increased with passage number starting from passage 3 indicating that the vaccine strain is well adapted to Vero cell environment and the virus replicates successfully to high titer as shown in Figure 2. The figure shows that as the passage number increases, the titer of the virus increases linearly. For example, in passage 3, the vaccine strain had a titer of $\log_{10}^{5.40}$ TCID/ml. According to Code of American Federal Regulation [23], IBD vaccine strain titer must not be less than $\log_{10}^{3.40}$ TCID₅₀/dose and the current titer ($\log_{10}^{5.40}$ TCID/ml) is higher than recommended by Code of American Federal Regulation [23] for protecting chickens against the disease. This suggests that the 3rd passage could be used as a vaccine to protect the chicken against vvIBDV. Previously, Hussain and Rasool [15] also reported similar findings in growth pattern of vvIBDV in Vero cells at passage 3 after 72 hours of infection. To confirm identity of the Vero-

cell adapted IBDV LC-75 vaccine strain, RT-PCR test conducted amplifying the VP2 region. As expected, we amplified and sequenced the expected 645 bp band of the VP2 gene fragment. Sequencing of the VP2 gene was recommended for checking absence of nucleotide and/or amino acid change since VP2 gene is responsible for the antigenicity [24], antigenic variation [25], and pathogenicity of the IBD virus [1]. Immune response status following vaccination was assessed using iELISA at 7 days post-inoculation, and all groups of chickens had contained antibody titer less than positive range (>0.3) suggesting that the vaccine might not provide sufficient protection within a week. Starting from day 7 up to day 14 post-inoculation, all groups of chickens inoculated with both Vero cells adapted virus and NVI-Gum 01/18 vaccine had increased antibody titer from 213.18 to 4220.74 or S/P (0.07 to 1.52). The mean antibody produced at day 14 post inoculations were closely similar with day 21 post-inoculation (7 days post-boost, Figure 4) suggesting as the maximum immune response was achieved but the exact day on which maximum immune response was achieved need to be determined by sampling the animals between days 14 and 21 and after 21 day. A similar idea was reported by Ahmad et al. [26] that the antibody titer at 14 days of post-infection resulted in a gradual increase of antibody values in all inoculated groups. This result also agreed with the report of Rautenschlein et al. [27] about immunity reaction to intermediate plus or "hot" vaccine of IBDV. As reported by Rautenschlein et al. [27] the intermediate plus vaccine-induced antibody levels already at 14 days post-vaccination. The mean S/P ratio was highly increased starting from day 21 post-inoculation. Due to that, all groups of chickens were boosted at day 14 post-inoculation, antibody production was enhanced and mean S/P ratio or the antibody titer increased. In all types of infections, there was no significant difference in antibody production. Therefore, Vero cell adapted IBDV vaccine strain IBDV is equally immunogenic at passages 5 and 10 with that of chicken fibroblast cell-based prepared vaccine. Upon comparative evaluation of the efficacy of the Vero cell adapted and CEF cell based vaccine against challenge with local field strain of vvIBDV, chickens vaccinated by the two cell-type based vaccines were fully protected with no mortality and morbidity. The challenged control chickens (unvaccinated groups) responded by high morbidity and mortality rate starting from 3rd day post-challenge. There were clinical manifestations of anorexia, ruffling of feathers and death on day 6 post-infection but no any clinical sign and death occurred in pre-vaccinated chickens. Therefore, Vero cell adapted virus is efficacious to protect the vvIBDV circulating in Ethiopia similar to that of CFC based prepared vaccine as described previously by OIE [13].

Conclusion

The present study confirmed that Vero cell adapted attenuated IBDV vaccine could be successfully produced similar with that of the CEF cell vaccine. The IBDV vaccine strain LC-75 was adapted well on Vero cells as early as passage 3 with a good virus titer/ml. The adapted vaccine is equally immunogenic and efficacious with no difference between passages and CEF cells based prepared vaccine. It is more economical to produce IBDV vaccine using Vero cells. Based on the above conclusions, production of IBDV vaccine using Vero cell line is recommended. In the future, a closely related cell type like avian stem cells or cell line developed from chicken could replace Vero cells and CEF cells for better vaccine

production of avian pathogens as it allows use of host-related cell type for vaccine production that overcomes adaptation problem and also facilitates better vaccine production.

Methods

Virus and cell culture

IBD virus intermediate plus LC-75 vaccine strain was used for the Vero cell culture adaptation. A challenge IBD virus, NVI/MB/263/17, with infectious titre $\log_{10}^{5.3}$ TCID₅₀/ml was used for assessing the protective potential of the vaccine. Vero cell, vaccine strain and challenge field virus isolate were obtained from the virology laboratory, Research and Development Directorate, NVI. Dulbecco's Modified Eagle's Medium (DMEM, HIMEIA) with 10% and 2% sterile fetal calf serum (FCS, Gibco) was used as a growth and maintenance medium for Vero cell culture, respectively. Media and solutions used for the growth of cells were Trypsin versine solution, phosphate buffered saline (PBS) and tryptose phosphate broth (TPB). Culture media were prepared according to the manufacturer's instruction (HIMEIA).

Adaption of vaccine strain using Vero cells

Vero cells were grown in 75cm² cell culture flask until confluent monolayer of cells was obtained. Confluent Vero cell monolayer was washed three times using prewarmed PBS pH7.4 to remove dead cells and suspended using pre-warmed 0.25% trypsin/EDTA solution. Cell viability was checked with trypan blue stain and the number of viable cells was counted using cell counter EVE™ Automatic cell counter (E16041- 016, NanoEnTekInc, Korea). The viable cells were counted and seeded into 75cm² tissue culture flask containing pre-warmed DMEM (Sigma-Aldrich) with sterile 10% fetal calf serum (FCS, Gibco) at a seeding density of 2.1×10^6 cells. The cells were incubated at 37°C with 5% CO₂. The cells were observed under inverted microscope for the formation of confluent monolayer [15].

Infectious Bursal Disease virus inoculation into Vero cell

Confluent monolayer of Vero cells at 36 hours after sub-culturing was washed three times using pre-warmed sterile PBS and used for virus infection using adsorption method. The cells were infected by adding 0.5 ml of IBDV LC-75 vaccine strain and incubated at 37°C for 1 hour with intermittent tilting of the flask to allow adsorption. After one-hour incubation, 10ml DMEM with 2% FCS was added into an infected flask and placed into 37°C incubator with 5% CO₂. One flask of fresh cells with confluent monolayer was kept as control under similar condition. The infected cells were observed daily for the development of virus induced cytopathic effects (CPE) for six days. At six days of post-inoculation the infected cells with the virus were harvested, labelled and stored at -80°C for 24 hrs. The harvested virus was freeze-thawed alternatively three times at -80°C/+30°C and inoculated to fresh monolayer of Vero

cells. This process was repeatedly conducted up to passage 10 (P10). At each passage, the harvested flasks were labeled and stored at -80°C until subsequent experiments.

Titration of infectious bursal disease virus

The viral suspension kept at -80°C was diluted 10-fold in sterile tubes (10^{-1} to 10^{-10}) of passages 2, 3, 5, 7, 9 and 10. Then 100µl viral dilutions were dispensed into 96 well micro plates containing 100µl Vero cells per well with ten replicates for each dilution. Column 11 was left empty and column 12 was inoculated only with cells to serve as negative control. Finally, the plate was sealed by micro plate sealer and incubated at 37°C in 5% CO₂. The inoculated plates were monitored under inverted microscope daily starting from 72 hours post-inoculation for eight days. The titre for each virus passage was determined according to Spearman Karber method [16].

Molecular characterization of IBD virus adapted in Vero cell

The identity of the IBD vaccine strain virus adapted in Vero cell was checked by amplification of the viral hypervariable core protein (VP2) gene of the IBD virus using gene-specific primers by reverse transcription polymerase chain reaction (RT-PCR) [17]. The VP2 gene amplification was done on vaccine working seed and from passages 1, 3, 5, 7, 9 and 10.

RNA extraction, cDNA synthesis and RT-PCR

Virus RNA extracted from the harvested virus using the RNeasy® Mini Kit (Qiagen) following the manufacturer's instruction. The extracted RNA was subjected to two steps RT-PCR. A 10µl reaction mix was prepared from 3µl RNase free water, 1µl Oligo dT, and 1µl of 10 Mm dNTP mix. Then, 5µl template RNA was added and incubated at 65°C for 5 minutes and placed at 4°C. A 10µl volume for cDNA synthesis mix was prepared from 1µl DEPC treated water, 2µl of 1X RT buffer, 4µl of 25 mM MgCl₂, 2µl of 0.1 MDTT, and 1µl superscript III-RT enzyme and incubated at 55°C for 50 minutes. The reactions were terminated at 85°C for 5 minutes and chilled on ice and then collected by brief centrifugation to which 1µl RNase was added per sample and incubated for 20 minutes at 37°C. Finally, the obtained cDNA was immediately used for PCR. For running PCR, a total of 20µl master mix was prepared by using 3µl of RNase free water, 2µl of forward primer, 2µl of reverse primer, 10µl of IQ super mix (Bio-Rad) and 3µl of template cDNA. The amplification forward and reverse primers used for the RT-PCR reaction to amplify the segment A, VP2 gene of the virus were used from the previously described method [18]. The two primers are expected to amplify 645 base pairs of the VP2 gene. Master mix was prepared and amplified using touchdown PCR (thermal cycler 2720, applied Bio-system). The PCR cycles involved initial denaturation at 95°C for 5 minutes, followed by 15 cycles of denaturation at 95°C for 30sec, annealing at 60°C for 30sec, and elongation at 72°C for 30sec. The 15 cycles of initial PCR followed by 20 cycles of denaturation at 95°C for 30sec, annealing at 56°C for 30sec, extension at 72°C for 30sec and final

extension at 72°C for 7 minutes for 1 cycle. The amplified PCR products were stained with GelRed® (Biotium) and visualized using 1.5% agarose gel electrophoresis in 1x TAE buffer. A 100 bp DNA ladder (Fermentas) was used and IBD virus positive samples yielded a PCR product of 645 bp band size.

Immunogenicity and efficacy tests

A total of eighty fourteen days old Koko breed specific pathogen free chicks of both sex were used for vaccine evaluation study. Chicks obtained from the Ethiopian Institute of Agricultural Research, Bishoftu and screened for presence of maternal antibody against IBD virus using indirect ELISA test (IDvet, France) before commencing the animal experiment. All maternal antibody free chicks aged fourteen-day were grouped randomly into four groups (group 1- 4); the sample size (number of chicks) per group were 20 chicks (total 80 chicks) according to OIE recommendation [13] to be included in one experimental group. The chicks were managed under the same animal facilities of separated room for each treatment group with similar management to avoid any confounding factors among the different groups. Group 1 and 2 were inoculated with Vero cell adapted IBDV passage 5 and 10, respectively. Group 3 chicks were inoculated with Gumboro vaccine produced based on chicken embryo fibroblast cell, Batch number NVI-Gum 01/18, for comparison of immunogenicity test of Vero cell adapted IBDV, and group 4 served as negative control. At 18 days of age chicks were inoculated using ocular route with $1 \times 10^{5.4}$ TCID₅₀ antigen from passage 5, 10 and from CEF cell based IBDV vaccine batch (Gum 01/18) which was the product of the NVI, Bishoftu, Ethiopia. The chicks were boosted at day 14 post-inoculation. Blood samples were collected from all treatment groups on day 7, 14, 21, and 28 post-inoculation and subjected to indirect ELISA for antibody detection and titration. Efficacy test was conducted by inoculating challenge virus, vvIBD-VMB/263/17 with titre $\log_{10} 10^{5.3}$ TCID₅₀/ml dose (0.2ml/chick) to vaccinated and non-vaccinated control chicken groups on 21 days post-vaccination by inoculating through eye drop route [19]. The challenged birds were observed for 10 days for morbidity and mortality and the results were recorded as per OIE recommendation [13]. The chicks were identified using unique identification number attached on the wing and grouped and follow up till data analysis blindly.

Indirect ELISA

Indirect ELISA test was done at the NVI following the manufacturers' instruction (IDvet, France). Optical density (OD) of the test result was read by using microtiter plate reader at 450nm absorbance. The percentage positivity for test samples in relation to the negative and the positive controls was calculated as per the formula given by the kit manufacturer. The result was interpreted with reference to the cut-off value recommended by the manufacturer as the OD_{PC} (positive control) should be >0.25, and OD_{PC}/OD_{NC} should be >3.0; formula indicated at the data management section.

Animals management

All experimental chicks handled according to the NVI animal handling and research ethics guideline. Feed and water provided ad libitum. After the end of the experiment, the chicks were euthanized humanly using manual cervical dislocation involved stretching and separating the cervical vertebrae by hand and properly incinerated using the incinerator located inside the institute compound considering all the biosafety and biosecurity measures. Ethical clearance obtained from the animal research ethical review committee, certificate Ref. No: AAU/CVMA/VM/ERC/28/05/10/2018.

Data management and statistical analysis

All the collected data were entered into Microsoft Excel spread sheet program and analyzed using STATA version 12 [20]. A one-way analysis of variance (ANOVA) was used to differentiate effect of passage levels on immunogenicity of vaccines in chicks. Statistical significance was based on $p < 0.05$. The formula used for calculation of sample antibody level was taken from the kit manufacturer as described below.

$$SP \text{ value} = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}}$$

Where; SP: sample to positive ratio, OD: optical density, NC: negative control, PC: positive control.

Abbreviations

CEF, chicken embryo fibroblast; CPE, cytopathic effects; DMEM, dulbecco's modified eagle's medium; iELISA, indirect enzyme linked immunosorbent assay; FCS, fetal calf serum; IBDV, infectious bursal disease virus; NVI, national veterinary institute, OD, optical density; OIE, world organization for animal health; PBS, phosphate buffered saline; SPF, specific pathogen-free; TCID, tissue culture infectivity dose; TPB, tryptose phosphate broth; VP2, viral hypervariable core protein; vvIBDV, very virulent IBDV.

Declarations

Ethics approval and consent to participate

Ethical clearance for this study was obtained from animal research ethics and review committee, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia (AAU/CVMA/VM/ERC/28/05/10/2018).

Consent for publication

Not applicable.

Availability of data and material

The sequence data are available at the National Center for Biotechnology Information (NCBI) (Genbank accession numbers: MK798159-61).

Competing interests

The authors declare that there is no conflict of interest.

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Authors' contributions

Conceived and designed the experiments: EG, MB, WK, FD, TA. Performed the experiments: WK, EG, HM. Analyzed the data: WK, EG, MB, FD, BM, BG, TA. Contributed reagents/materials: WK, EG, MB, BG, TA, MY. Wrote the paper: WK, EG, MB, FD, BM, TA. Supervised the study: EG, FD. Edited the final manuscript: EG, MB, FD, BM, BG, TA, MY. All authors have read and agreed to the published version of the manuscript.

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Author details

¹National Veterinary Institute, PO Box: 19, Bishoftu, Ethiopia. ²Ethiopian Biotechnology Institute, PO Box: 32853, Addis Ababa, Ethiopia. ³College of Veterinary Medicine and Agriculture, Addis Ababa University, PO Box: 34, Bishoftu, Ethiopia

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Figures

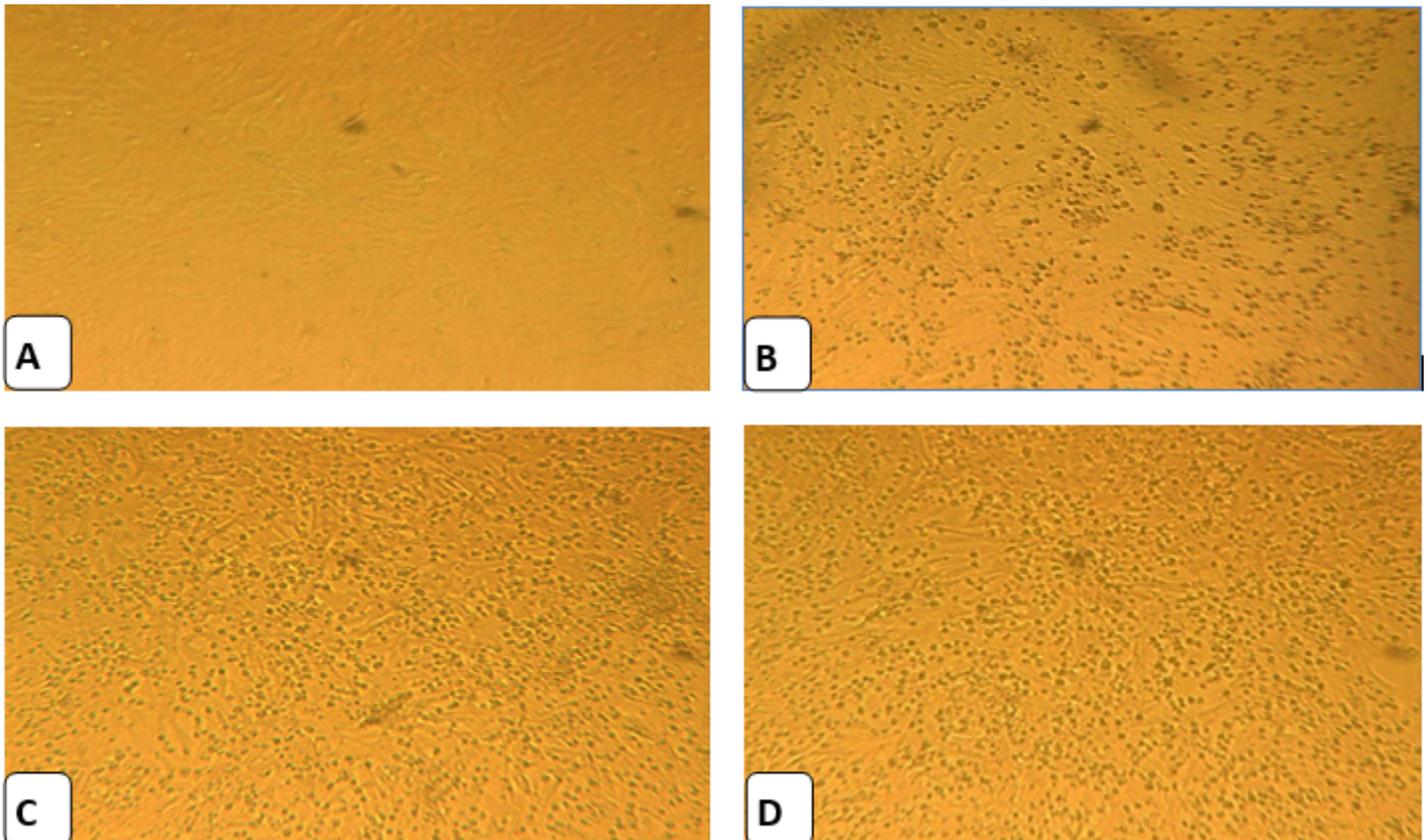


Figure 1

Cytopathic effect (CPE) observed on Vero cell culture infected at different passages of IBDV LC-75 vaccine strain using Inverted microscope (40x). Where; (A) Vero cell control, and observation of CPE were recorded at (B) passage 5, (C) passage 7, and (D) passage 9.

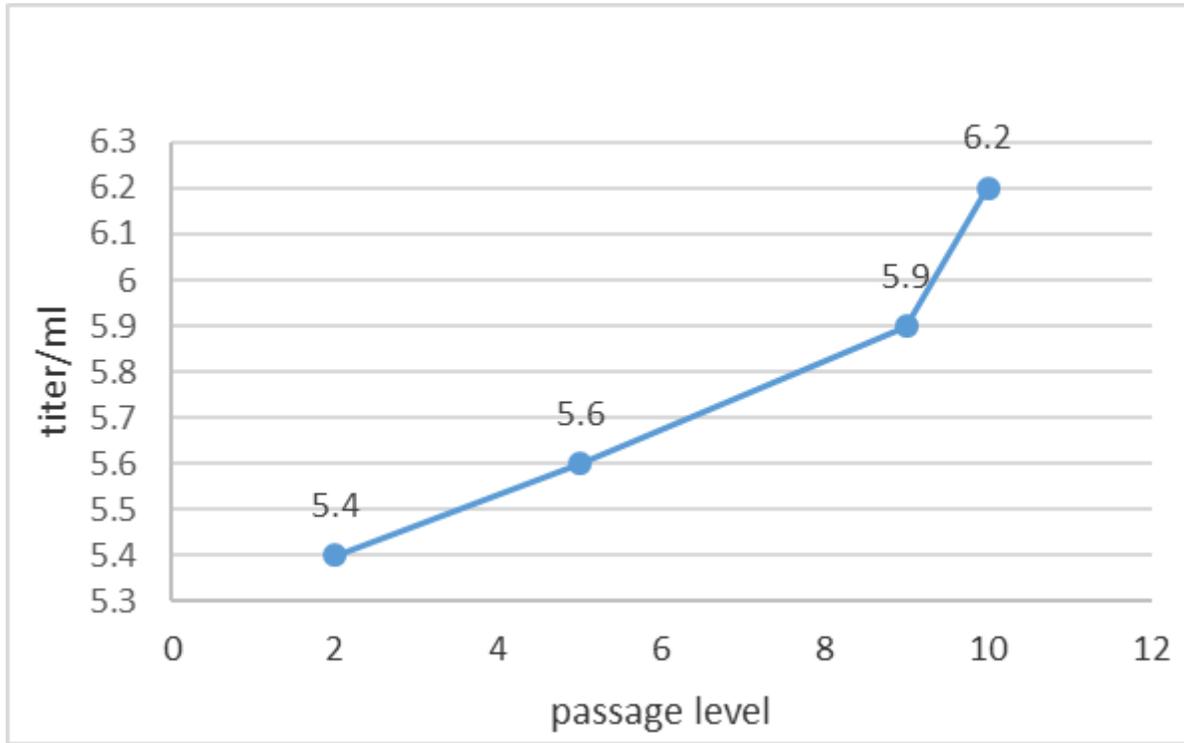


Figure 2

Vero cell adapted IBD virus titration result at different passage number.

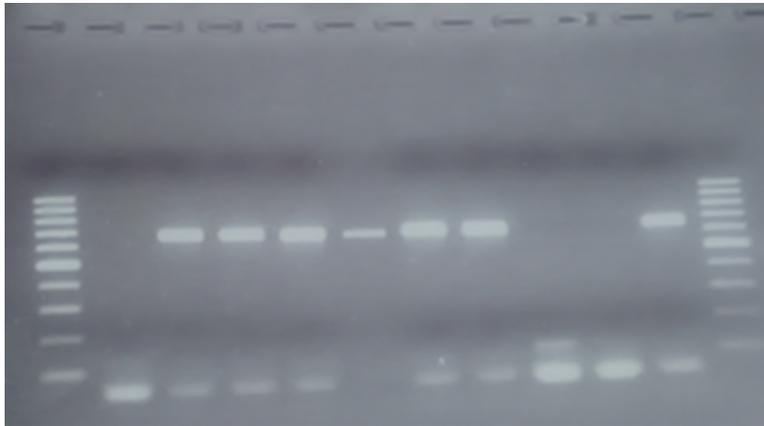


Figure 3

Agarose gel electrophoresis picture showing the 645 bp fragment of the IBDV VP2 gene. Where; (M) Molecular ladder (100bp, Fermentas), (1) Vero cell control (Negative), (2) Original culture (Positive), (3) Vero cell culture passage 5, (4) Vero cell culture passage 6, (5) Vero cell culture passage 7, (6) Vero cell

culture passage 8, (7) Vero cell culture passage 9, (8) Extraction control (RNA free water), and (9) Negative control.

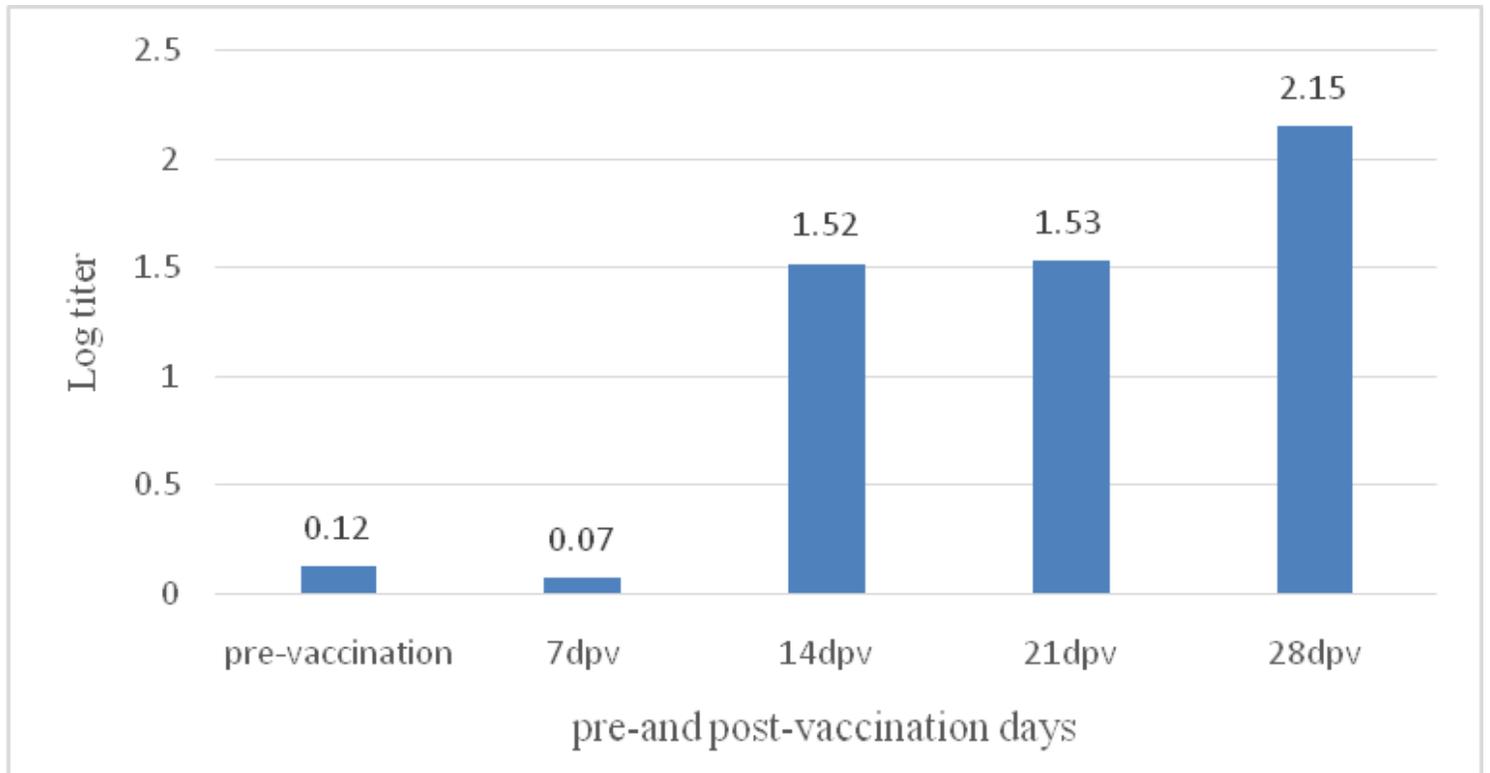


Figure 4

Mean S/P ratio of specific IBDV antibody at pre-and post-vaccination. dpv: days post-vaccination.

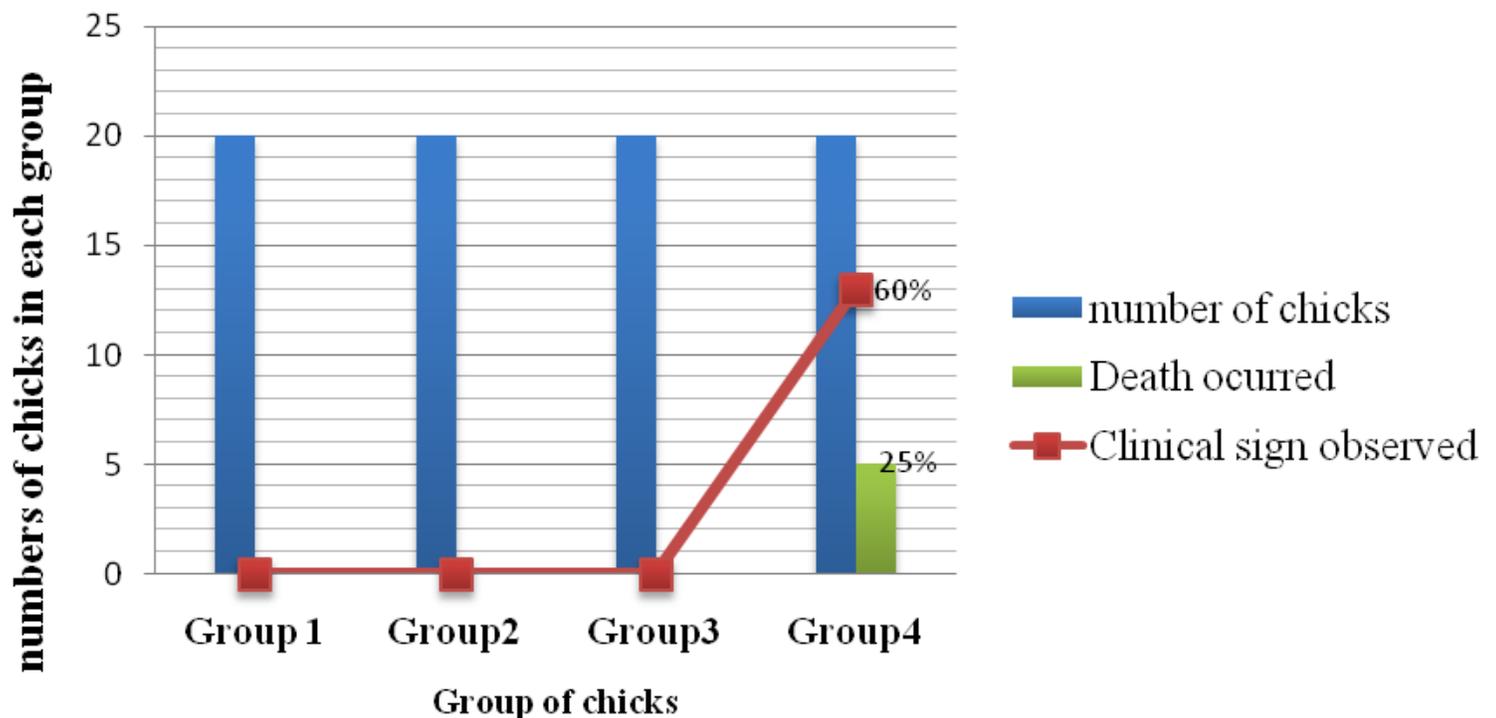


Figure 5

Percentage of clinical sign and death observed in chicken vaccinated with different vaccine passages and challenged with vvIBDV.

Supplementary Files

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