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*Full Length Research Paper*

# **Factors associated with infectious bursal disease vaccination failure in Dar es salaam, Tanzania**

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**Infectious bursal disease (IBD) is a viral infection that affects young chicks. The IBD outbreaks in vaccinated chickens are reported in Tanzania frequently. The current study was conducted to find out the possible causes of vaccination failure focusing on knowledge and practices of vaccine sellers and users, the immunogenic potential of currently used vaccine and the phylogenetic relationship between the vaccine and the field strains. A cross-sectional study was performed to gather information on knowledge and practices from 384 poultry farmers and 20 veterinary outlets in Dar es Salaam. Results revealed inadequate knowledge of farmers in vaccine handling and administration and also breaches in the cold chain maintenance by vaccine sellers was apparent. A total of 60 chicks were experimentally vaccinated with Virgo 7 strain vaccine and titers of induced antibodies assessed. The vaccine induced adequate antibodies against IBDV, confirming its immunogenic efficacy. Isolated nucleic acids from the vaccine and field strains were sequenced and result shows that IBDV field isolates, are genetically different from the vaccine strains used in the country. The field isolates belong to the vvIBDV African types, while the vaccines belong to the vvIBDV European/Asian or classical virulent types. Putting together results from this study reveals multiple possible reasons which may contribute to vaccine failures. These include poor vaccine handling by farmers and vaccine sellers and the genetic disparity between the field and vaccine strains. It is therefore recommended that veterinary regulatory authorities should ensure good vaccine handling practices and considering local virus isolates during vaccine development.**

**Key words:** Infectious bursal disease (IBD), Infectious Bursal Disease Virus (vvIBDV), phylogenetic analysis, strains, vaccine, poultry farmers and antibodies.

## **INTRODUCTION**

Infectious bursal disease caused by Infectious Bursal Disease Virus (IBDV) is an acute viral infection which is

extremely contagious and affects growing chickens between the age of 3 to 6 weeks (Swai et al., 2011). The

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disease is also called “Gumboro disease”, because the first case occurred in the Delaware town of Gumboro, USA, (Cosgrove, 1962). The main clinical signs manifested by diseased chickens include; watery diarrhoea, immunosuppression hemorrhagic syndrome, depression, dehydration, inappetence, ruffled feathers, vent picking and reluctant to rise (Chawinga, 2016). The IBD virus affects more B cells in an immature stage and therefore induces immunosuppression (Meulemans, 2000). Since the chickens become immunosuppressed, they also build up a weak immune response when vaccinated against other diseases (Müller et al., 2012). The infectious bursal disease is therefore considered as one of the setbacks in poultry farming around the world (Etteradossi and Saif, 2008).

The IBD virus belongs to the family *Birnaviridae* under the genus *Avibirnavirus* and is none enveloped. It is double-stranded RNA and bi-segmented virus, that is, segment A and B (Etteradossi and Saif, 2008). There are two IBDV serotypes reported worldwide namely, serotype I and II; however, it was reported that only serotype I contains pathogenic strain to chickens. The serotype I is further categorized as very virulent (VV), mild, intermediate, classical virulent strains, and antigenic variants (Ching et al., 2007).

For a long time, IBD has been reported to be one of the major hindrance factors for the poultry farming globally; however, the disease has become more devastating especially in the last two decades after the appearance of very virulent and variant strains (Mutinda, 2016). The first report of infectious bursal disease in Tanzania came from Dar es Salaam and the coastal regions in 1988 (Kasanga et al., 2007). After that, the disease spread all over the country in poultry farming units that led to significant economic losses to farmers (Jenberie et al., 2014). The main effective way to control IBD is vaccination and different vaccination programmes are regularly implemented globally including Africa. However, there has been a vaccination failure in different parts of the globe (Berg, 2000). Studies showed that vaccination time, maternal antibodies in the chicks, vaccine type, and virulence of local strains are the main factors that determine the vaccine effectiveness (Hair-Bejo et al., 2004). In several African countries, including Tanzania, the majority of vaccines used for the control of IBD are imported; this may result in vaccine failure due to antigenic differences between circulating local IBDV isolates and vaccine strains (Adamu et al., 2013). In this regard the IBDV strains included in the imported vaccines should be considered if they are contiguous with field IBDV isolates.

The farmers in Tanzania use different types of IBDV vaccines and vaccination programs; however severe IBD outbreaks are still reported in vaccinated chickens caused high mortalities hence becomes a priority problem (Kasanga et al., 2007). The same problem has

been reported from other countries like Nigeria where a severe IBD outbreak was encountered even in vaccinated chickens (Musa et al., 2010). Since the IBD outbreaks reported in vaccinated, this indicates an inefficiency or failure of vaccine used in the country (Kasanga et al., 2007). No research has been carried out to investigate the possible causes of vaccine failures; even though farmers reported IBD outbreak in vaccinated chicken flocks. Thus, the current study aimed to examine the possible causes of vaccination failure including characterization of field IBDV strains and vaccine strains, assessment of immunogenic potential of the Virgo 7 intermediate hot strain IBD vaccine (VIR-114) commonly used in chicks in Tanzania and the KAPs of vaccine sellers and poultry farmers towards managing IBD vaccine and vaccination.

## MATERIALS AND METHODS

### Knowledge and practices of vaccine sellers and poultry farmer in IBD

A cross-sectional study was conducted in Dar es salaam, Tanzania and covered commercial poultry farmers and veterinary outlets that render services to these farmers, including selling vaccines. The study was conducted between March and May 2019. A structured questionnaires and checklist were used, and physical inspection was conducted to collect data on IBDV from 384 poultry farmers and 20 veterinary outlets. The study size was arrived by using a formula for cross-sectional studies of Kish and Lisle (1965). The interviews focused on schedules of vaccination, maintenance of cold chain, transportation, storage, reconstitution and administration of the vaccine and general IBD management. Also, data on occurrences of IBD cases in vaccinated chickens and brands of IBD vaccines used by farmers were captured. Analysis of data was performed by using Statistical Package for Social Science (SPSS) version 20. The data collected during the study were processed using Microsoft Excel after that it was imported into SPSS version 20. Frequencies for all the variables were calculated, means and standard deviations were computed for the continuous variables. Knowledge, attitude and practices were calculated by adding the scores from the variables regarding the respective rating. Chi-square test and simple linear regression were used to measure the association between several factors. P-value  $\leq 0.05$  with 95% CI was considered to be significant.

### Study on the immunogenicity of Virgo 7 strain vaccine

Sixty (60) day-old broiler chicks were purchased from a private hatchery (Mkuza farm) in the Coastal region and raised at Sokoine University of Agriculture (SUA) farm. The intermediate plus Virgo 7 strain vaccine manufactured by Biovac Ltd, Israel was bought from the veterinary outlets. The chicken house was thoroughly cleaned, washed, disinfected with Virid® and maintained for two weeks prior introduction of chicks to prevent any possible infections. Regardless of the sex, chicks were randomly allocated into two groups of 30 chicks each. Group one was vaccinated, and group two was not vaccinated and served as unvaccinated control. Furthermore, chicks were given anticoccidial (Amprolium) drug at day 7 for five days and antibiotics for 5 days to prevent early infections. Chicks were wing-tagged for individual identification. Water and feed were

provided ad-libitum. The handling of chicks was done according to the Tanzania Animal welfare act, 2008.

Blood samples were collected from the wing vein of the chicks at specific intervals up to day 42. Approximately 0.5 ml of blood was collected from each chick at day one old to determine the maternally derived antibodies (MDAs) titer for IBDV. At day 14 of age chicks were sampled and vaccinated orally via drinking water as per the manufacturer's instructions. In addition, 0.5 ml of blood was collected at days, 21st, 28th, 35th, and 42nd of age to obtain sera. Blood samples were left to form clots in a slanting position at room temperature overnight. Each blood sample was centrifuged for 15 min at 3000 rpm to separate the sera. The serum was harvested by using Pasteur pipettes and transferred into Eppendorf tubes. All sera were stored at -20°C until analysis.

Serology test was done by using indirect ELISA kits (ID Vet, France), as per the manufacturer's directions. A single serum dilution of (1:500) was used for the detection of IBDV specific antibody. Briefly, the ELISA kit reagents and serum samples that were stored at 2-8°C and at -20°C respectively were allowed to thaw at room temperature before the test. Five micro liters (5 µl) of each sample to be tested were added to the microtitre plate. Then dilution buffer-14 was added to all wells except to control wells A1, B1, C1, and D1. The negative control was added to wells A1, and B1 and positive control were added to wells C1 and D1 of the ID.vet test kit plate. Another dilution buffer-14 was added to all wells except to control wells A1, B1, C1, and D1. Then 10 µL of the pre-diluted samples was added to each corresponding well of the ID.vet test kit plate. The plate was incubated for 30 min at a temperature of 27°C. After 30 min, the plate was washed three times, with 300 µl of wash solution. Then the conjugate was added to each well. The plate was covered and incubated for 30 min at a temperature of 27°C. After 30 min, the plate was washed as above. The substrate solution was added to each well. The plate was covered and incubated for 15 min at a temperature of 27°C. After that, each well was added with 384 µl of stop solution to stop the reaction. The absorbance values were read and reported at wavelength 450 nm using ELISA reader, which is Multiscan TM FC Microplate Photometer (Thermo Scientific TM). The antibody titer in chicken serum samples was calculated by referring to the positive control. That association was expressed as S/P ratio (Sample to Positive Ratio).

The interpretation of the results was made based on the S/P ratio and ELISA antibody titer. The result was positive if the S/P ratio is >0.3 or antibody titer >875 and the result was considered negative when the S/P ratio is ≤0.3 or antibody titer ≤875. Calculation of the S/P ratio and the antibody titer was done by Microsoft Excel 2007.

Serological data were analyzed by using IBM SPSS Statistics 20 for descriptive statistics. A Student t-test was used for comparison of antibody titer between vaccinated and non-vaccinated groups. One-way analysis of variance (ANOVA) was employed to evaluate the differences in mean titers between groups at each sampling time and the overall.

#### **Characterization of field IBDV strains and the vaccines currently used in the region study samples**

The IBDV samples used in this study were obtained from the repository based at Sokoine University of Agriculture (SUA). The samples were obtained during IBD outbreaks in Morogoro (n=2) and Dar es Salaam (n=1) between 2016 and 2018. Two commercial live IBDV vaccines, namely; Virgo 7 intermediate strain (Biovac Ltd, Israel) and Globivac- Intermediate plus strain (India) were purchased from the veterinary outlet located in the study area.

#### **IBDV RNA extraction and cDNA synthesis**

The viral RNA was isolated from the bursal of Fabricius of the confirmed diseased chickens and vaccines using Viral RNA Min Extraction Kit (Qiagen, West Sussex, UK). The extraction was done as per the manufacturer's instructions and followed by cDNA synthesis. The cDNA strand was manufactured by using a commercially available cDNA kit (RevertAid First Strand cDNA Synthesis Kit) manufactured by Thermo Scientific, Lithuania EU. Briefly, 3 µl of each extracted dsRNA was mixed with 1.5 µl of dimethyl sulfoxide (DMSO) and incubated at 97°C for 5 min then quickly chilled on ice. The RT master mix, which contains reaction buffer, ribolock, random primer, revert Aid, dNTP's and nuclease-free water was added to the tube containing RNA-DMSO mixture to the final volume of 22.5 µl. The mixture was thoroughly mixed, and the tubes were then sealed and centrifuged to spin down the contents and eliminate any bubbles. The sample mixture was then subjected to thermal cycler under the following conditions; 25°C for 10 min, 37°C for 120 min and 85°C for 5min. The synthesized cDNA was kept at 4°C and used as a template for PCR.

#### **Polymerase chain reaction**

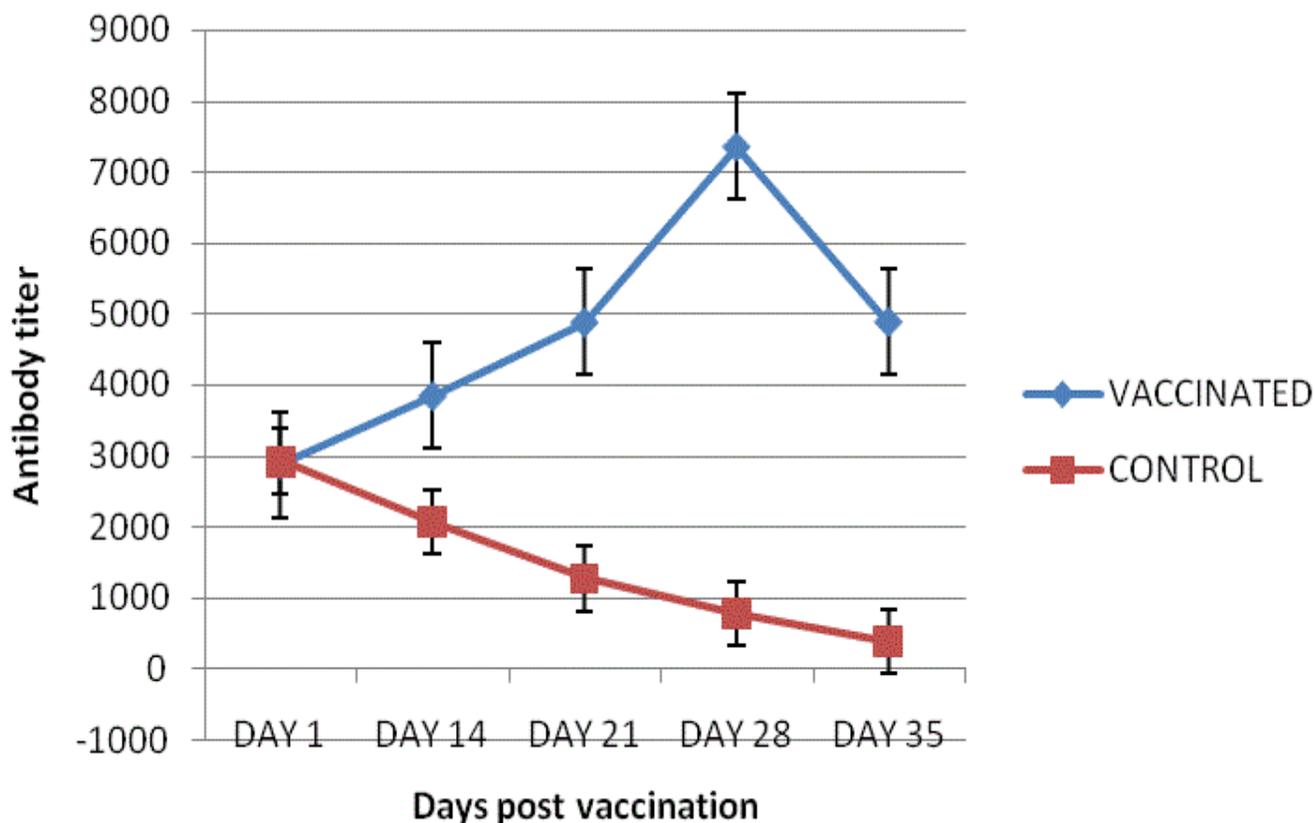
Polymerase chain reaction (PCR) was conducted by targeting the VP2 HVRs of IBDV through gene-specific V1 forward primer (5-CCA GAG TCT ACA CCA TAA-3) and V2 reverse primer (3-CCT GTT GCC ACT CTT TCG TA) (Yamaguchi et al., 1996). The process was done by using Bioneer PCR PreMix® readymade Kit (Bioneer Corporation, South Korea). The PCR was carried out at initial denaturation at 97°C for 5 min, followed by 35 thermal cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, elongation at 72°C for 30 s and final elongation step at 72°C for 5 min. The PCR products were confirmed by electrophoresis in 1.2 g agarose gel supplemented with 3 µl Gel red.

#### **Nucleotide sequencing and phylogenetic analysis**

Sequencing was done at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture by using AB 3500 Genetic analyzer. The sequencing was conducted as previously described (Sanger et al., 1977). Nucleotide sequences were assembled and edited using Genius software, version 4.0.10. Confirmation of identity and homology were performed by using BLAST (Basic Local Alignment Search Tool). Phylogenetic analysis was performed by using MEGA7; the accuracy of the tree was estimated from the bootstrap values using the Kimura two-parameter option with 1000 bootstrap replicates created by the neighbor-joining (NJ) method (Kimura, 1980). A phylogenetic hierarchy was created by using nucleotide sequences of the study samples (strain PDSM18, MMRG16, RMRG19, Virgo 7 strain, and Globivac) and VP2 gene sequences from 32 IBDV strains with various genotypes taken from GenBank were used (Figure 1). The nucleotide accession number of the samples and the reference strains obtained from GenBank are shown in Table 1.

#### **Ethical approval**

The study got approval from the Northern Zone Health Research Ethics Committee (KNCHREC) with permit number KNCHREC0013. All the participants involved in the study were provided with a pre-informed consent form to express their willingness to participate in the study.



**Figure 1.** Antibody titer after administration of Virgo 7 strain vaccine compared to unvaccinated controls.

**Table 1.** List of IBDV isolate for comparison of VP2 gene of segment A Genome.

Virus strain	Accession no	Origin	Genotype
BJ836	AF413069.1	China	Classical
IBD071R	KT633995.1	Iran	Classical
534-North-Carolina	MF142546.1	USA	Classical
IBDV76/NVR1	JX424075.1	Nigeria	Classical
D78	EU162087.1	USA	Classical
03-27950-dn	EF138988.1	Canada	Classical
GBF-1	D16828.1	Japan	Classical
JG011/KEN/15	KY407590.1	Kenya	VV-EU Type
213-048-2	KY556581.1	Brazil	VV-EU Type
713-Russia	MF142562.1	Russia	VV-EU Type
KM523629.1	IBD10HLJ02	China	VV-EU Type
West Bengal/HBL-07-15-b	KT630855.1	India	VV-EU Type
V90/TW95	JQ315162.1	Taiwan	VV-EU Type
IBDV78/ABIC vaccine	IBD10HLJ02	Russia	VV-EU Type
UK661	KT630855.1	China	VV-EU Type
D6948	JQ315162.1	India	VV-EU Type
KMRG-40	AB200982.1	Tanzania	VV-A Type
KARS-53	AB200984.1	Tanzania	VV-A Type
KMZA-78	AB200985.1	Tanzania	VV-A Type
LUSC47-2016	LC319700.1	Zambia	VV-A Type

## RESULTS

### Knowledge and practices of poultry farmers on IBD management

#### *Demographic characteristics of study participants*

A total of 384 poultry farmers in Dar es Salaam city were interviewed with more than half (66%) of the respondents being females who proportionally owned more flocks. The respondents had a mean age of 42 years (Std 9.3 years). The majority of the respondents (89%) were the owner of the project, hence aided in the provision of right information regarding the control of the infectious bursal disease. The flock size varied between 170 and 4000 chickens per household. More than half (53%) of the poultry keepers had between 5 and 8 years of poultry farming experience with the mean years of experience being  $5.20 \pm 3.14$  years.

About 68% of the respondents had secondary school education and above. The majority of the respondents were commercial poultry farmers (78%), followed by employees (13%) and others (9%). Of all the respondents interviewed, 73% kept broilers, 21% kept layers and 6% local breeds.

#### *Respondents' knowledge on IBD*

The majority of the respondent (91%) heard of infectious bursal disease (IBD). Although 91% of the respondents heard about the disease, 65% were not aware of the causative agent. In addition, a small proportion (14%) of the respondents knew the IBD mode of transmission with clinical signs such as white watery diarrhea, leg paralysis, and sudden death frequently reported.

Generally, the respondents appeared to have poor knowledge on IBD causative agent, mode of transmission and symptoms. Furthermore, gender ( $p=0.024$ ), education ( $p=0.014$ ), flock size ( $p=0.021$ ) and time for the keeping of chickens ( $p=0.01$ ) had significant influence on poultry keeper's IBD knowledge.

#### *Respondents' attitude toward IBD management*

All (100%) respondents agreed that maintaining a high level of bio-security measure help in preventing the spread of IBD. Majority (86%) of the respondents believe that IBD can be prevented by vaccination and 14% were neutral. A high proportion (89%) of respondents reported that proper vaccine handling including cold chain maintenance is important for vaccine effectiveness. About two-thirds (66%) of respondents did not know if the presence of disinfectants in water interferes with vaccine function.

Generally, the respondents appeared to have positive attitude toward IBD with a mean score of  $4.68 \pm 1.18$  out of 6. On top of that education ( $p=0.03$ ), flock size ( $p=0.02$ ) and time for the keeping of chickens ( $p=0.01$ ) have a significant influence on poultry keepers' attitude toward IBD.

#### *Respondents' practices which may contribute to IBD vaccines failure*

Despite the fact that all respondents did routine cleaning, only 50% cleaned on weekly basis, followed by 20% who did once per batch and 30% without a routine schedule for cleanness. Based on vaccine handling, all respondents admitted that vaccines are usually packed in plastic bags with ice packs for transport from veterinary shops to farms. Although 91% of respondents vaccinate against IBD, 19% experienced outbreaks of IBD (Table 2). Ninety percent (90%) of respondents who experienced IBD outbreaks after vaccination did not adhere to the entire recommended procedures for vaccine reconstitution, handling, and biosecurity measures. More than two-thirds (78%) of respondents vaccinate their chickens once using the IBD vaccine whereas 22% vaccinate twice. It was also observed that water from different sources was used to reconstitute the IBD vaccine, that is, tap water (50%), well water (48%) and tap water mixed with skimmed milk (2%). Only 31% of the respondents placed a foot bath at the entrance of the poultry house.

#### *Handling of a vaccine in veterinary shops*

All the 20 veterinary outlets owners who were interviewed reported packing vaccines in plastic bags with ice packs for their customers. Fourteen out of 20 outlets had a standby generator and, the remaining outlets either store their vaccines in cool boxes with ice packs or doing nothing during periods of a power outage. It was noted that the majority (14) of the shop operators lacked formal training in maintaining or handling vaccines, especially the maintenance of the cold chain. All shops did not have written procedures for packing vaccines into cold boxes or vaccine carries. There was neither temperature recording chart nor thermometer for monitoring the temperature in all the veterinary shops visited.

#### *Immunogenicity of Virgo 7 strain vaccine*

Following the administration of the Virgo 7 strain vaccine, the mean serum antibody titers in the vaccinated group started to increase which peaked on 3<sup>rd</sup> week (day 35 of age). Compared to unvaccinated group, the mean serum

**Table 2.** Practices related to IBD management in Dar es Salaam region.

Variable	Frequency (n)	Percentage
<b>Disinfection of poultry house and equipment</b>		
Yes	384	100
No	0	0
<b>How often do you clean the chicken house, feeder and drinkers</b>		
Every week	192	50
Once per batch	77	20
No specific schedule	115	30
<b>Do you have a foot bath on the entrance of the poultry house?</b>		
Yes	119	31
No	265	69
<b>Do you vaccinate your chicken</b>		
Yes	349	91
No	35	9
<b>Vaccination regimen for IBD?</b>		
Once	272	78
Twice	77	22
Others	0	0
<b>How do you store vaccine before vaccination</b>		
Store in refrigerator	35	10
Use immediately	314	90
<b>Transportation of vaccine from the shop to farm</b>		
Packed plastic bag with ice	349	100
Packed in cool box with ice	0	0
<b>Which water do you use in reconstituting the vaccine?</b>		
Tap water	174	50
Well water	167	48
Tap water + milk	8	2
<b>Have you ever experience IBD after vaccinating the chickens</b>		
Yes	70	20
No	279	80

antibody titers in the vaccinated group were significantly higher ( $p < 0.05$ ) from day 14, all through day 35 (Figure 1). Antibody titers in control group progressively decreased and were at lowest levels on day 42 when the experiment was terminated. This was due to the diminishing of maternal antibodies.

The variation of mean antibody titer between age group in vaccinated chickens was analyzed by one way ANOVA and found to be statistically significant ( $p = 0.0001$ ). Furthermore, titers from vaccinated and non-vaccinated groups were compared using paired independent t-test

and the result shows a significant variation of antibody titers ( $p < 0.005$ ) at 21<sup>st</sup>, 28<sup>th</sup>, 35<sup>th</sup> and 42<sup>nd</sup> day.

### **Molecular characterization of commercial IBD vaccines and field samples**

#### ***BLAST search analysis of VP2-HVR***

On BLAST search (available from GenBank), the nucleotide sequences of two Tanzanian IBDV field

samples (PDSM18 and MMRG16) were highly similar (94.68-96.33% nucleotide sequence identity) to a virus (LUSC47-2016) detected in Zambia 2016. Sample RMRG19 showed 99.53% nucleotide sequence identity with G028/KEN/16 isolated in Kenya. Globivac vaccine showed 99.34% nucleotide sequence identity with D78 isolated in the USA. However, Virgo 7 strain vaccine showed a 100% nucleotide sequence identity with strain IBD10HLJ02 isolated in China, 713\_Russia isolated in Russia, 213-048-2 isolated in Brazil, West Bengal/HBL-07-15-b isolated in India and V90/TW95 isolated in Taiwan.

### **Phylogenetic analysis**

Phylogenetic analysis using the hyper-variable region of the VP2 gene of the field IBDV samples, vaccines and the representative strains from GenBank was performed to establish the genetic and antigenic relationship of IBDV and examine the evolutionary lineage of the study samples. The phylogenetic trees revealed that the IBDV samples were separated into two major groups, namely; Classical virulent (cv) and very virulent (vv) viruses (Figure 2). The very virulent (VV) IBDVs further divided into two major groups, named VV1 and VV2. VV1 contains strains derived from Africa including Tanzania, Zambia, Kenya and Nigeria which are called the vv IBDV African genotype, while VV2 contains strains isolated from various parts of the world including Africa, Europe, Asia and America which form vvIBDV European genotype. The current field strains (RMRG19, PDSM18, and MMRG16) were grouped in the VV1 cluster while the imported Virgo 7 strain vaccine was grouped in VV2 and Globivac vaccine was grouped in classical virulent type (Figure 2).

Phylogenetic tree based upon nucleotides of segment A showed that the field IBDV strain PDSM18 and MMRG16 are closely related (96% bootstrap value) with vvIBDV African type (LUSC47-2016). The field strain RMRG19 was more associated (66% bootstrap value) with both vvIBDV African types (JG028/KEN/16 and JG026/KEN/16). The imported Virgo 7 strain vaccine was genetically closely related (97% bootstrap value) to the vvIBDV European/Asian type JG011/KEN/15, 213-048-2/2017, BD10HLJ02, 13\_Russia-2017, V90/TW95, and IBDV78/ABIC. The imported Globivac vaccine was genetically related to (100% bootstrap value) classical virulent (D78, 534\_North\_Carolina, 03-27950-dn, and BD07IR). The sequence analyses indicated that the field isolates were genetically different from the vaccine strains present in the market for IBD control.

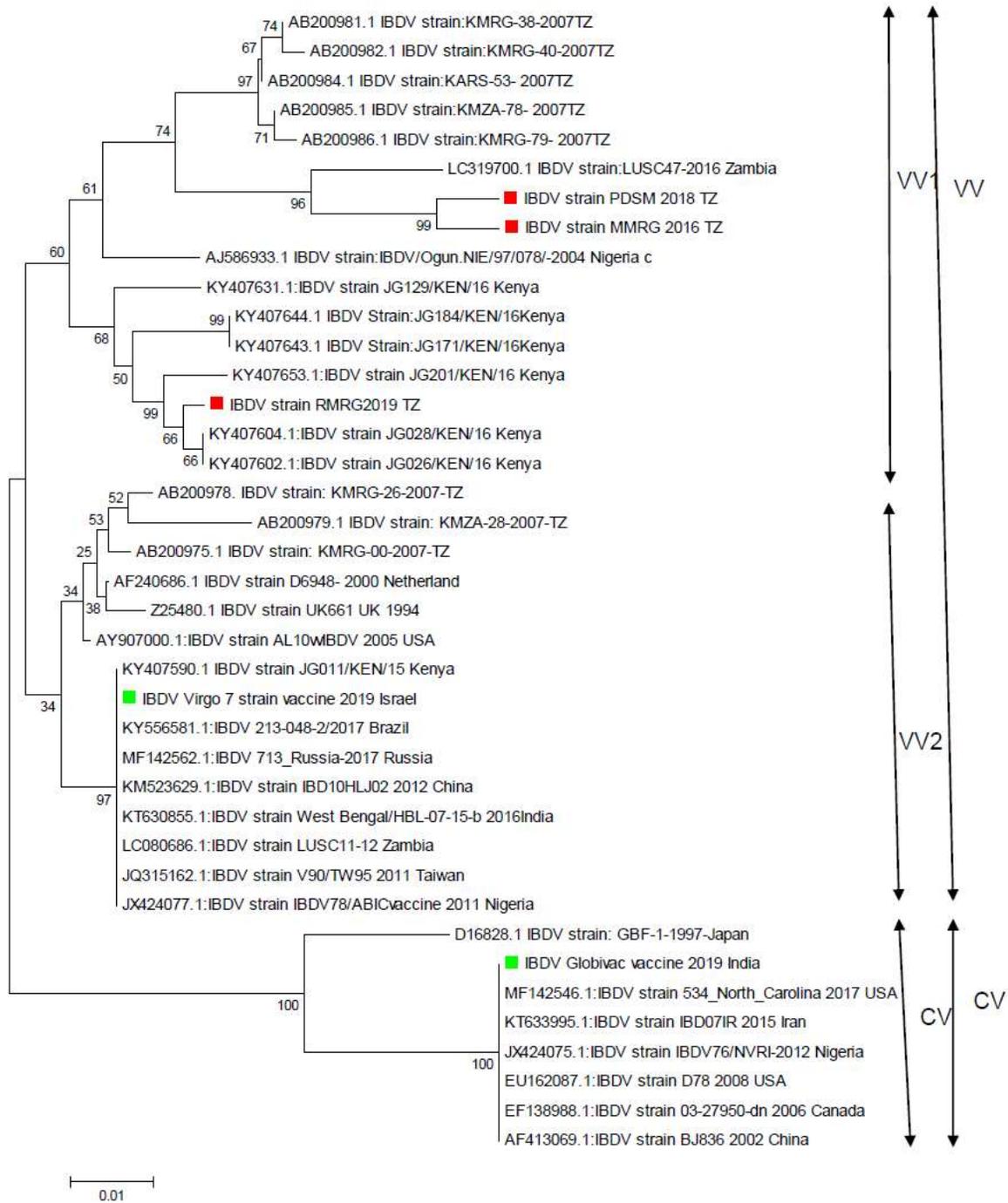
## **DISCUSSION**

Infectious bursal disease is a very devastating disease of

poultry due to mortality and rendering chickens to be susceptible to secondary infections (Kurukulasuriya, 2017). The disease is more severe in developing countries since the suitable vaccines that would control the IBDV field strains effectively are lacking (Mohamed et al., 2014) and inadequate biosecurity measures. In Tanzania, some poultry farmers have reported IBD outbreaks in both vaccinated and unvaccinated chickens, suggesting vaccine inefficiency or failure (Kasanga et al., 2007). Therefore, the current study was performed to examine the possible causes of vaccination failure focusing on assessment of immunogenic potential of the Virgo 7 intermediate hot strain IBD vaccine (VIR-114) in chicks, knowledge, attitude, and practices of poultry farmers and vaccine sellers and phylogenetic relationship between circulating field IBD strains and imported infectious bursal disease virus vaccine strains.

To evaluate the knowledge, attitude and practices of poultry farmers, a structured questionnaire was used. Results demonstrated that the majority of poultry farmers had heard about the infectious bursal disease in their locality. However, despite previous knowledge of the disease by the majority of farmers, they were still unaware of details of the disease such as causes, transmission and clinical signs similar to reports from other countries (Chawinga, 2016; Radostits et al., 2000). The knowledge gap identified in farmers with regard to causes, signs, and transmission against IBD is an indication of poor poultry extension services on IBD management. Therefore, there is a need for strengthening poultry extension services and training to poultry farmers on disease management, especially IBD. These extension services and training will improve the farmer's knowledge and subsequently lower IBD transmission rates.

In contrast, the study conducted by Wahome (2018) reported a better awareness about IBD among poultry keepers in Embu Kenya. The variation in awareness and knowledge on the clinical presentation of IBD, transmission, and causes may be attributed to the endemic rate of IBD in the community, presence of veterinary services, formal training to poultry farmers and education through other media in Embu County, Kenya. Those few farmers who had a fair knowledge of IBD could be due to their habits of consulting veterinary officers, whenever they see problems in their flocks and being exposed to the media where they get information on several diseases. Even though knowledge of IBD was low among poultry farmers, the level of education was found to influence farmer's knowledge against IBD. Those with a high level of education were observed to be somehow knowledgeable compared to those with a low level of education. This could be due to the fact that educated farmers are more likely to understand disease management practices. A similar observation has been reported in other studies (Isegbe et al., 2014) which show



**Figure 2.** Phylogenetic tree of 3 fields IBDV isolates reference strains (cv and vv) and imported vaccines. Phylogenetic tree calculated by the neighbor-joining (NJ) method based on the hypervariable domain of the VP2 capsid gene of IBDV serotype 1 strains using nucleotide alignment created in MEGA version 7. The numbers at forks indicate the bootstrap values (1000 replicates) in the important junction of the tree.

that farmers with a high level of education are most likely to understand recommended vaccine instruction and disease management.

Flock size and duration of keeping chickens also reported influencing farmer’s knowledge against IBD in this study. This could be for the reason that when farmers

keep a large number of chickens, they are more likely to consult their neighbors and veterinary personnel before vaccination and when there is disease outbreak due to fear of loss. Furthermore, when farmers keep chickens for a long time, they are most likely to encounter a number of diseases, so they become aware of so many diseases.

A structured questionnaire was also used to assess the practices of the poultry farmers, and the results showed that 91% of respondents vaccinate their chickens against IBD. The findings were similar to the study carried out in Nigeria, which showed that 97.7% of respondents vaccinated their birds (Nongo and Bosh, 2004). However, IBD outbreaks have been observed in vaccinated flocks in this study, as also reported in Kenya and Sudan (Babiker et al., 2008; Kasanga et al., 2007; Mutinda, 2016). This reported vaccination failure might be due to the consequences of violations of the prescribed vaccine handling and administration procedures demonstrated by the farmers which include poor biosecurity measures, non-adherence to cold chain system, vaccine preparation and administration. The other factor could be due to the appearance of a very virulent IBDV that differs antigenically from the virus used in the vaccines, as reported in this study. All IBDV vaccines used in Tanzania are imported from other countries with a different serotype. The same finding has been reported in Sudan, where 51% of mortality that occurred in vaccinated chickens was caused by vvIBD (Yousif, 2005).

Majority of the respondents reported that during vaccine reconstitution, they use potable water that contains chlorine although IBD vaccine manufacturers recommended the use of chlorine-free water or if doubtful, add 2 g/L of skim milk. The addition of skimmed milk in water used for vaccination is very important as it helps to counteract the effects of chlorine and other dissolved chemicals that can impair the vaccine's effectiveness. Therefore the practice of using potable water by a majority of the poultry farmer in the study area might be one of the causes for observed incidences of vaccination failure. This high rate of farmers using chlorinated water in vaccine reconstitution was also reported in another study whereby only 18% of farmers use chlorine-free water (Isegbe et al., 2014).

The present study also assessed the status of cold chain in veterinary outlets in Dar es Salaam region by using a checklist. The results showed that there was a lack of functional thermometer in all veterinary shops surveyed. As per the vaccine logistic management guidelines, the thermometer must be available in any vaccine storage center to record the maximum and minimum temperature. This guarantees vaccine potency and the success of immunization, when it is not available at vaccine storage centers. That means such centers do not monitor their refrigerator temperature and in that

case, the cold chain can easily be interrupted. The interruption of the cold chain might affect the antigenicity of the vaccine hence resulting in vaccination failure. The finding is in agreement with Azira et al. (2014) and Simba and Msamanga (1994) who emphasized temperature monitoring in vaccine centers to ensure vaccines are of acceptable quality until being administered to recipients. Another issue of serious concern was the availability of electricity power. Some of the outlets visited do not have a backup generator in case of a power outage. This gives an indication that cold chain maintenance is interrupted at some point and exposing the vaccines to unfavorable temperature. It should be noted that electricity or an alternative source of power is essential for cold chain maintenance as high temperatures may cause the death of viruses and affect the potency of the vaccines as reported by Nongo and Bosh (2004) and subsequently cause vaccine failure. A similar result was also reported in Enugu State, Nigeria (Joy, 2013).

An experimental study was conducted to evaluate the ability of commonly used IBDV vaccine (Virgo 7 intermediate hot strain IBDV vaccine) to induce antibodies against IBDV following vaccination of chickens. The results showed that Virgo 7 intermediate hot strain IBDV vaccine was capable of producing antibodies against IBDV in vaccinated chickens. Based on the results obtained in the experimental study it is recommended that vaccination will be successful if implemented after two weeks of age where MDA level will be below the protection level and has less interference with live vaccines. However, farmers and other stakeholders should note that vaccination time depends on the source of chickens and the type of vaccines used. Different breeder farms have a different level of MDA (Hassan et al., 2018) hence should have different vaccination schedule. The current study has shown that poultry farmers in Dar es Salaam use the same vaccination schedule regardless of the source and type of chicks; this might be one of the factors that account for the incidences of IBD outbreaks in vaccinated chicken flocks.

On the other hand, this study should be conducted on a wider scale so as to confirm the potency of other IBD vaccines being used in the country. Further study is also needed to examine the protective efficacy of this and other IBD vaccines being used in Tanzania.

Phylogenetic analysis of three field strains and two imported vaccine strains has been conducted to assess their genetic relatedness. Phylogenetic study of the deduced nucleotide sequences of the three Tanzanian field strains showed that all samples belong to the pathogenic serotype-1, whereas subgenotype analysis revealed that the samples belong to the vvIBDV African genotypes, which include strains from the west, east and southern Africa. The current Virgo 7 strain vaccine used in Tanzania belongs to the IBDV European/Asian

genotype, whereas the Globivac vaccine belonged to the classical strain. This indicates that both vaccines used in the control of IBDV in Tanzania are genetically different from the field strains circulating in the country and this may contribute to outbreaks in vaccinated flocks. This calls for further research on appropriate vaccines made using local IBD strains as previously recommended (Mannan et al., 2015).

Furthermore, other studies have demonstrated that vaccination of chicken using vaccine made by classical virulent IBDV strains to prevent the disease caused by very virulent IBDV strains might cause exchange of genes between the two viruses and resulting in a natural re-assortant virus as reported in China, Zambia and Argentina (Fernandes et al., 2012; Kasanga, 2015). In view of this understanding, the basis for evolutionary characteristics and antigenic variation of field viruses could help veterinarians and researchers to design and develop new vaccines for controlling IBD, which cannot be controlled by current vaccines being used in the country.

Generally, vaccine failures have been reported globally (Adamu et al., 2013; Müller et al., 2012) and are attributed to several factors. Under the current study, the observed attributed factors for vaccination failure were poor biosecurity measures, lack of adherence to vaccination regimen, use of chlorinated water in vaccine reconstitution, cold chain breaks due to frequent power outage, lack of standby generator and using vaccines with different strains from circulating field strain in Tanzania. Those factors may compromise the vaccine efficacy, and vaccination process hence resulted in reported cases of IBD outbreaks in vaccinated chicken flocks. These findings are comparable to other studies done earlier (Chawinga, 2016; Mor et al., 2010; Mutinda et al., 2014) that the underlying causes of high morbidity and mortality rates were improper vaccination, poor sanitation, non-adherence to recommended vaccine storage temperature, fewer drinking facilities, emergence of antigenically different field strains from vaccine strains, presence of chlorine in drinking water and water used to reconstitute vaccines.

## CONCLUSION AND RECOMMENDATIONS

The success of vaccination programme depends on various factors like vaccine composition, route of administration, timing in administering a vaccine, the ability of the chickens to produce antibodies after vaccination and the adherence of vaccine handlers/users to the manufacturer's recommendation. In the current study, the knowledge of vaccine handling and administration was observed to be inadequate among both vaccine sellers and farmers. The present study pointed out that there were a lot of breaches in the cold chain maintenance, which may possibly jeopardize the

efficacy of the vaccines and the overall quality of the vaccination services. Therefore, poultry extension services should be strengthened to educate farmers and vaccine handlers (veterinary outlets) on the importance of observing all recommended vaccine protocols to minimize vaccine failure in the country. Veterinary regulatory authorities and extension services should ensure good vaccine handling practices by vaccine sellers and appropriate vaccine use by poultry farmers. It is recommended to provide training on vaccine handling and administration through various media such as using electronic media services, workshops, and meetings prior to vaccinations.

The current study indicated that maternal antibodies against IBDV are passively transferred to the progeny. Therefore early vaccination may cause neutralization of vaccine viruses by circulating MDA. The current study proposed vaccination using Virgo 7 strain vaccine to be done at day 14<sup>th</sup> of age as proposed by vaccine manufacturer since the vaccine was able to produce antibodies against IBDV in vaccinated chickens. However, further studies are needed to investigate whether the induced antibodies by study vaccine are protective against Tanzania IBDV strains. Also further research is recommended to address other factors that may influence time to vaccinate, such as a source of chicks and type of vaccine and to confirm the potency of other IBD vaccines being used in the country apart from Virgo 7 strain vaccine. The current study discovered genotypic differences between Tanzanian IBDV isolates and imported vaccine strains (Globivac and Virgo 7 strain). This study suggested considering local virus isolates during vaccine development, and further investigation is warranted to establish transmission dynamics, evolutionary characteristics and antigenicity of IBDV in order to design appropriate control strategies.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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