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Isolation and Molecular Detection of Newcastle Disease Virus from Outbreaks cases in Chicken in Selected Districts of Western Ethiopia

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Abstract

Newcastle disease is the challenging and highly contagious poultry viral disease globally by causing great economic losses in the poultry industry. An outbreak investigation was conducted from October 2020 to May 2021 to detect and isolate *Newcastle disease virus* from chicken in selected districts of western Ethiopia. A total of 56 chickens were sampled in 20 pooled samples collected from five outbreaks.Samples were obtained from clinical cases and postmortem procedures. All samples were tested by rRT-PCR and positive samples were subjected to virus isolation using CEF DF-1 cells. Of 20 pooled samples tested by rRT-PCR, 11 (55%) were positive of *Newcastle disease virus*. Among 11 positive samples, 72.72% were tissue samples, and 27.27% were swab. All tissue samples were 100% tested positive. Samples collected from Gaba Jimata, Bako, Tibe Gedo and Babich showed positivity rates 50%, 50%, 100%, 66.67%, and 0%, respectively. From the 11 rRT-PCR positive samples inoculated on DF-1 cells, 10 (90.9%) were successfully isolated and showed cytopathic effects on cells, including cells swelling, death and monolayer disruption. This study showed that Newcastle disease is a problem in the study area, affecting both commercial and backyard chickens. Therefore, routine intensive care, awareness creation and effective vaccination of chicken are recommended to overcome the problem.

1. Introduction

The poultry industry has confronted many socioeconomic crises throughout the world from different infectious diseases. Viral diseases in poultry like Newcastle disease have a devastating economic effect on global domestic poultry production. Newcastle disease is a fatal as well as top-ranked poultry disease which has the annual losses of millions of dollars worldwide [1, 2]. In Ethiopia, poultry production is the key livestock subsector that plays a great role in generating work opportunities. It is a suitable business for poor households due to the small quantity of land needed and the low investment cost required to start-up and running the business. The poultry population of Ethiopia is estimated to be around 60 million and about 56% of Ethiopian households have poultry holdings with a variable range of flock size [3].

Despite its role in raising incomes and reducing poverty in local communities in Ethiopia, poultry production is hampered by wide ranges of constraints among which infectious diseases are common. Newcastle disease, infectious bursal disease, mycoplasmosis, pasteurellosis, and salmonellosis are infectious diseases that mostly affect the poultry industries [4]. Newcastle disease is an important infectious disease of the poultry that is caused by virulent strains of Avian Paramyxovirus-1 (APMV-1), known as Newcastle diseases virus (NDV), a single strand non-segmented negative-sense Ribonucleic Acid (RNA) virus. It is a reportable disease to World Organization for Animal Health (OIE) and also endemic in many countries in Asia and Africa [5].

About 250 avian species are reportedly susceptible to natural or experimental infection with NDV and many more species might be susceptible [6, 7]. More commonly affected species are chickens, turkeys, ducks, pigeons with the most susceptible avian species to this disease are chickens [8, 9]. The

paramyxoviruses isolated from avian species have been classified by serological testing and phylogenetic analysis into ten subtypes designated APMV-1 to APMV-10 [10, 1]. There are two major divisions of NDVs; class I and class II. Class, I NDV is less divergent and belongs to a single genotype, whereas class II isolates are more diverse [11]. All NDV strains belong to a single serotype but significant genetic diversity observed in different NDV isolates. Class, I strains are mostly avirulent and mainly isolated from wild birds and waterfowl birds whereas class II contains low virulent (lentogenic), intermediate virulent (mesogenic), and high virulent (velogenic) strains [12, 13]. Class II is further divided into 18 genotypes, I-XVII [14]. In Ethiopia isolates of NDV were assigned to Class II and genotypes VI [15, 16]. and genotype VII [15].

Newcastle disease is one of the most important diseases responsible for the devastating outbreaks in poultry flocks and a substantial ongoing threat to domestic and wild bird populations in Ethiopia [16]. Newcastle disease is the dominant poultry viral disease-causing major problem in poultry production by huge economic losses especially in countries having recently developing poultry industries like Ethiopia [17]. Even though the disease has a prominent effect on the economy, studies conducted on isolation and molecular exploration of this viral disease was inadequate when compared to its effect and no published paper was available on isolation and molecular detection of the NDV in western Ethiopia. This study was designed based on the repeated occurrence of the outbreaks from year to year that imposed huge losses in poultry production both in commercial and backyard chickens in the study area. There was reports of sick chickens having respiratory, nervous and gastro intestinal problems in different farms, which is suggestive of NDV and needed to be confirmed by the laboratory works. There molecular works for confirmation of Newcastle disease suspected cases in the study area was difficult and chickens were simply treated by using tentative diagnosis. So, to confirm the case with appropriate diagnosis of the virus was needed to overcome the problem by letting some information for both farmers and the body in responsibility. The objective of this study was to isolate and molecularlt detect NDV from chickens outbreaks in selected districts of western Ethiopia.

2. Materials and Methods

2.1 Study Area

The study was conducted in selected districts of western Ethiopia where NDV outbreaks had been reported. Outbreaks occurred in Wayu Tuka district of East Wollega zone and Bako Tibe, Chalia, and Liban Jawi districts of West Shewa zone as indicated on Fig. 1. From these districts, outbreaks were recorded in Gaba Jimata (Wayu Tuka district), Bako and Tibe (Bako Tibe district), Gedo (Chalia district), and Babich (Liban Jawi district) where samples were collected during the study period. According to recent data obtained from Central Statistical Agency, East wollega zone has a total population of; 1,008,968 Cattle, 274,629 Sheep, 189,644 Goats, 4,030 Mules, 121,636 Donkeys and 770,250 Poultry. West Shewa zone has 2,202,544 Cattle, 1,048,490 Sheep, 313,569 Goats, 264,969 Horses, 7,143 Mules, 237,825 Donkeys and 1,273,221 Poultry [18]. Geographically, the study sites were located between 170 to 315 km to the west of the capital, Addis Ababa.

2.2 Study Population

The study populations were chickens of all ages, breeds, and sexs, reared under both backyard and commercial production systems,that were suspected of NDV case during outbreak investigation in the study area. Priority for sample collection was given to chicken showing visible clinical signs of Newcastle disease and tissue samples were collected from severely affected chickens.

2.3 Study Design and Sampling Technique

The study focused on the detection of NDV from suspected cases during an outbreak investigation conducted from October 2020 to May 2021. The study population was purposively selected based on reports of the outbreak. Selected chickens were physically examined for any signs suggestive of NDV infection (diarrhea, ocular and nasal discharge, respiratory distress, nervous signs, or sudden death).Severely affected (near death) chicken were selected for postmortem examination and collection of tissue samples. Data on sampled chickens like; site, age, breed, production type and vaccination history, were obtained from owners by short interviews during sample collection.

2.4 Sample Size

A total of 20 pooled samples from 56 chickens were collected from five outbreaks for this study (6 pooled cloacal swabs, 6 pooled tracheal swabs, and 8 pooled tissue samples). From 56 chickens sampled for this study, 13 chickens were from Gaba Jimata (2 pooled cloacal swabs, 2 pooled tracheal swabs and 4 pooled tissue samples), 13 chickens were from Gedo (1 pooled cloacal swabs, 1 pooled tracheal swabs and 4 pooled tissue samples), 10 chickens from Bako (1 pooled tracheal and 1 pooled cloacal swabs), 10 chickens from Tibe (1 pooled tracheal and 1 pooled cloacal swabs), and 10 chickens from Babich (1 pooled tracheal and 1 pooled cloacal swabs). Of the 56 sampled chicken, 20 were from backyard and 36 were from commercial production systems. Regarding age, 20 chicken were above 2 months old and 36 chicken were less than 2 months old. Interms of breed, 10 were local and 46 were exotic breed. Vccination status indicated that 20 chicken were unvaccinated and 36 were vaccinated.

2.5. Methodology

2.5.1 Sample Collection and Transportation

Samples were collected purposively from clinically affected chickens in each outbreak in the study area during the study period. Cloacal and tracheal swabs were collected by inserting sterile cotton-tipped swabs deeply into the vent and trachea, respectively, and gently swabbing walls.The swabswere then transferred into sterile, labeled, and 2 ml cryovials. Tissues samples from the brain, lung and trachea, intestine, and other internal organs (pooled tissues) were also collected from recently dead chicken and transferred into sterile labeled falcon tubes. The collected samples were transported under cold chain in an icebox containing ice packs to the Animal Health institute(AHI) for isolation and molecular detection. Viral transporting media (VTM) containing PBS with antibiotic and antimycotic was used for samples transportation. Upon submission to AHI the samples were stored at -80°C until processed [19].

2.6 Laboratory Investigations

2.6.1 Molecular detection of NDV

The real-time Reverse transcription-Polymerase Chain Reaction (rRT-PCR) method is quick, sensitive and has recently been used for the identification and typing of NDV [20]. In this study ,rRT-PCR was used to detect the presence or absence of NDV in samples collected during outbreak from the study area. It can detect the virus at very low concentration, sensitive and provides results in a short period when properly operated.

Sample Preparation

Samples collected and transported in VTM, stored at -80°C were transferred to 4°C refrigerator overnight to be thaw. The working area (biosafety cabinet class II) was disinfected using 70% alcohol to avoid contamination.Tissue samples were transferred from the falcon tube to a pre-sterilized mortar and a small amount of phosphate buffered saline (PBS) containing antibiotics was added. The tissues were chopped into small pieces using sterilized scissors to facilitate grinding.A small amount of sterile sand was added and the tissues were homogenized using a sterilized pestle. The homogenate was transferred to the labeled cryovials for molecular detection of the virus. Both processed tissue samples and swab samples were centrifuged briefly at 8000 rotation per minute (rpm) for 1 minute.

Viral RNA Extraction

Viral RNA extractions from tracheal, cloacal swabs and tissue samples was conducted using Qiagen® viral RNA Mini kit according to the manufacturer's instruction [21]. The extraction consisted four steps; lysis, binding, and two washing steps (Wash 1 and Wash 2) and elusion, which were conducted to obtain purified viral RNA.

Reverse Transcription Polymerase Chain Reaction (rRT-PCR)

Real-time RT-PCR was performed using a one-step RT-PCR kit (Qiagen, Germany) according to manufacturer instruction. Swab samples were processed first, followed by tissue samples. The master mixture used for both swab and tissue samples and the amount needed for a single reaction were; 6.45 µL Nuclease-free water, 5 µL 5× One-step RT-PCR Qiagen buffer, 1.25 µL $MgCl_2$, 0.8 µL dNTPs, 0.5 µL forward primer, 0.5 µL reverse primer, 0.5 µL inhibitor, 1 µL enzyme mixture and 1 µL probe. The total volume of master mix required for all samples was calculated based on the number of reactions.

For each reaction, 17 µL of the master mixture was combined with 8 µL of template RNA. Nuclease-free water (25 µL) was used as a negative control and 8 µL of known NDV positive controls was included as positive control.One-step rRT-PCR targeting the conserved region of matrix gene was performed using an Applied Biosystems 7500 fast Thermocycler (softwere version 2.3) to detect NDV. The sequences of the forward and reverse primers, as well as probe used for the amplification of the matrix gene,are indicated

in Table 1. The time and temperature used for reverse transcription, initial denaturation (pre-amplification), denaturation, annealing, and extension during amplification of matrix-gene are indicated in Table 2.

Table 1
Primers and probe used in real-time RT-PCR

Primers and Probe	Sequences
M+ 4100F	5'-AGTGATGTGCTCGGACCTTC-3'
M-4220R	5'-CCTGAGGAGAGGCATTTGCTA-3'
M + 4169	5'-FAM-TTCTCTAGCAGTGGGACAGCCTGC-TAMRA-3'

Table 2
Amplification program used for real-time RT-PCR

S/N	Step	Temprature	Time	Cycle
1	Reverse transcription	50°C	30 minutes	
2	Initial denaturation	95°C	15 minutes	
3	Denaturation	94°C	10 seconds	40 cycles
4	Annealing	52°C	30 seconds	
5	Extension	72°C	30 seconds	

2.6.2 Viral isolation

Cell Preparation

Immortalized chicken embryo fibroblast (CEF) cells (DF-1, passage 41), were revived from liquid nitrogen.One milliliter of cell suspension was diluted in nine milliliter of Dulbecco's Modified Eagle's Medium (DMEM) and centrifuged at 1500 rpm for 10 minutes. The pellet formed at the bottom and the supernatant was removed. Again 10 mL of the DMEM was added and the suspension was dispensed into a 25 cm^2 flask and incubated at 37^oC in a humidified incubator with 5% CO_2 until the cells multiplied well. After the cells grew well, the medium was removed and the cells wer washed three times with PBS to completely remove traces of serum that could inhibit trypsin activity. Then, two milliliter of trypsin was added to the side of the flask opposite to the cells, ensuring the monolayer was completely covered, and incubated for two minutes,checking every 30 seconds for the monolayer detachment.The action of trypsin was ceased by adding the medium. Any undetached cells were detached from the wall and from each other by repeated pipetting up and down over the surface bearing the monolayer,with the pipette tip resting on the bottom corner of wells without creating a foam. Finally, the cell suspensions was checked under an inverted microscope, and 0.5 ml of the suspension was dispensed into a 24 wells plate and incubated at 37^oC in a humidified 5% CO_2 incubator [22].

Sample Preparation

Samples stored at -80^{o}C were transferred to a 4^{o}C refrigerator overnight to melt. Tissue samples were removed from the VTM and chopped into small pieces using sterile scissors. The chopped samples were further minced by a mortar and pestle using sterile sand to facilitate maceration and washed several times with PBS containing antibiotics and antimycotic. The suspensions were freeze-thawed three times between room temperature and – 20 ^{o}C. Then, a 10% suspension was prepared in sterile PBS supplemented with 2% antibiotic-antimycotic solution, transferred to a centrifuge tube, and centrifuged at 3000 rpm for 20 minutes. For swab samples, the swabs were macerated well in the VTM used for collection. The resulting suspensions were centrifuged at 3000 rpm for 20 minutes. The supernatant was collected and filtered by 0.45 ml Millipore filters to reduce contamination (especially bacterial contamination) into new and labeled cryovials. The filter samples were then prepared for inoculation into suitable cell lines [22].

Inoculation of the Virus into DF-1 Monolayer Cells

Monolayer cell cultures prepared in 24 well plates with greater than 70% confluence were selected and the growth medium (complete medium) was removed. The cell culture was washed twice with 0.5 mL PBS. Then, 0.1 mL of the specimen suspensions was inoculated into labeled wells of the test plate, while PBS with antibiotics and antimycotic (without any specimen inoculums)was added to negative control wells.The test plate was incubated at 37^{o}C for 60 minutes to allow the virus to adsorption onto the cell culture. Then, 0.5 ml of maintenance medium (MEM with 2% Fetal Calf Serum) was added to each specimen wells and the test plate was incubated at 37^{o}C in a humidified incubator with 5% CO_2 for 6 days. Cells were monitored every 24 hours post-infection and inspected for CPEs using an inverted microscope and the effects were recorded.

Second Passage, the cell culture was prepared similar to the first one.The first infected cells were freeze-thawed three times between room temperature and – 80^{o}C and transferred to labeled cryovials. After centrifuged at 3000 rpm for 10 minutes, 0.1 ml of the supernatant was added to the newly prepared cell culture. Then, incubated at 37^{o}C for 60 minutes for adsorption and 0.5 ml of medium added to each well, labeled and incubated at 37^{o}C in a humidified incubator with 5% CO_2 for 5 days. CPEs were monitored every 24 hours using an inverted microscope and the effects were recorded. **Third Passage**, cell culture was prepared similar to the first and the second and specimen transfer was performed as in the second passage. The procedure was followed,except that the third passage was incubated only for 3 days and monitored every 24 hours for any changes and CPEs. Then, the CPE of positive samples were recorded and interpreted.

2.7 Data Management and Analysis

Data obtained from the the interview questionnaire and laboratory analyses were entered into a Microsoft Excel spreadsheet and appropriately coded. Information collected during sample collection

such as district, outbreak site, age, breed, production type, sample type and vaccination history was organized. All statistical analyses were conducted using STATA 14 (Stata Corp, College Station, TX, USA). Descriptive statistics were computed for all variables. A univariable logistic regression model was used to describe the data and explain the relationship between the dependent variable and independent variables.

3. Results

3.1 Detection of NDV using Real-Time RT-PCR

From the 20 pooled samples (12 pooled swab samples and 8 pooled tissue samples) detected by real-time RT-PCR, 11 (55%) were positive for NDV. From the four districts investigated for the outbreak of ND, samples from three districts (Wayu Tuka, Bako Tibe, and Chalia) were tested positive with the highest detection rate (75%) observed in Bako Tibe district. Of he five outbreak sites sampled in the study area, four sites (Gaba Jimata, Bako, Tibe, and Gedo) were confirmed positive by real-time RT-PCR, with Tibe showing a 100% positive rate. However, samples collected from Liban Jawi (Babich) were negative by real-time RT-PCR as indicated in Table 3.

This study also indicated that exotic breed chicken, backyard production system and unvaccinated chickens were more affected. The virus was highly detected in the pooled tissue samples 100% (8/8) than swabs samples (Table 3).

Table 3
Proportion of real-time RT-PCR test result with variables

Variables	Category	No of Pool tested	No +	%	P-value
District	Wayu Tuka	8	4	50	0.321
	Bako Tibe	4	3	75	
	Chalia	6	4	66.67	
	Liban Jawi	2	0	0.00	
Site	Gaba Jimata	8	4	50	0.341
	Bako	2	1	50	
	Tibe	2	2	100	
	Gedo	6	4	66.67	
	Babich	2	0	0.00	
Age	< 2 months	16	8	50	0.369
	≥ 2 months	4	3	75	
Breed	Local	2	1	50	0.881
	Exotic	18	10	55.56	
Production type	Commercial	16	8	50	0.369
	Backyard	4	3	75	
Sample type	Tissue	8	8	100	0.001
	Swab	12	3	25	
Vaccination history	Vaccinated	16	8	50	0.369
	Not vaccinated	4	3	75	

Table 4
Univariable logistic regression models for variables and real-time RT-PCR result

Variables	Category	OR	Std. Err.	95% CI	P-value
District	Wayu Tuka	Ref			.660
	Bako Tibe	2.99	4.06	[.21, 42.62]	
	Chalia	2.00	2.23	[.22, 17.89]	
Site	Gaba Jimata	Ref			.807
	Bako	1.00	1.58	[.04, 22.17]	
	Gedo	2.00	2.23	[.22, 17.89]	
Age	< 2 months	Ref			.357
	> 2 months	3.00	3.77	[.25, 35.33]	
Breed	Exotic	Ref			.881
	Local	.800	1.19	[.04, 14.88]	
Production type	Commercial	Ref			.357
	Backyard	3.00	3.77	[.25, 35.33]	
Vaccination history	No	Ref			.357
	Yes	.33	.419	[.02, 3.92]	
OR: Odds ratio, Ref: Reference category, CI: Confidence interval, Std.Err: Standard Error					

Tibe site and sample type were ommitted due to 100% detection rate (Complete success)

Figure 3. Real time RT-PCR amplification plot showing amplification with respective of swab samples and controls

3.2 Isolation of NDV on DF-1 cells

From isolation of 11 pooled samples (2 pooled cloacal swabs, 1 pooled tracheal swab, and 8 pooled tissue samples) which were detected positive by real-time RT-PCR and isolated on DF-1 cells, 10/11(90.9%) of them were detected positive for NDV on CEF DF-1 cells. From 4 outbreak sites detected positive real-time RT-PCR (Gaba Jimata, Bako, Tibe, and Gedo) and inoculated on DF-1 cells for isolation, all samples from these outbreaks were showed CPE and detected positive for viral isolation. From 4 pooled samples detected positive for real-time RT-PCR and inoculated on CEF DF-1 cells from Gaba Jimata, 3 (75%) were positive for virus isolation. All real-time RT-PCR positive samples inoculated from Bako, Tibe, and Gedo were 100% detected positive for virus isolation.

The infectivity in the cell line was not observed in the first inoculation and was sluggish in the second passage. During the first inoculation of cells that was monitored every 24 hours post-infection; there was no contamination observed and the monolayers were normal and have not shown any sign of detachment of monolayers. In the second passages, even if the virus did not produce a clear effect, there were some changes from the negative controls like cell swelling and cell death, especially after the fifth day of the second inoculation. However, in the third passage, the monolayers have been started to detach within a few days post-inoculation and clear CPEs have been started to be visualized. The negative controls were also observed for the occurrence of any change. The viruses caused changes in characteristics of cell monolayers such as clear cell swelling, cell death, cell rounding, cell detachment from monolayers, and disruption of monolayers observed as indicated in Fig. 3.

4. Discussion

This study indicated that the virus presents both in backyard chickens and commercial farms in outbreaks which indicates ND is the major problem in poultry production causing a great effect on the economy of farmers and farm owners in the study area. As indicated by [23], ND is an economically important disease and a major threat to the poultry industry. The clinical and postmortem findings observed during sample collection in this study were incoordination, greenish diarrhea, paralysis of the neck, legs and wings, swelling of eyes, necrotic lesions in proventriculus and hemorrhage in different internal organs especially on intestines. This findings were also reported by previous researchers such as; [24] in Iraq and [25] in Brazil.

In this study, from 20 pooled samples 11 (55%) were detected positive of NDV by real-time RT-PCR. This finding was relatively agreed with the previous study in different parts of Ethiopia like; 39.2% (188/480) in Northwestern Ethiopia [26] and 55.8% (121/217) in Western Amhara [27]. The result obtained from real-time RT-PCR might be affected by factors such as any technical errors in laboratory or sampling errors like selection biases, sampling time and availability of virus shedding during sample collection.

This study indicated that both commercial and backyard chickens have been affected by NDV even if a higher detection rate (75%) occurred in backyard chickens production than commercial type production system (farms). This result was agreed with the study conducted by [16], which stated that NDV can freely move between the two bird populations; commercial poultry farms and village chickens due to poor biosecurity practices in commercial farms and live poultry markets. According to [28], ND is known to cause heavy losses in village chickens as compared to commercial poultry farms in Ethiopia. As stated by [29], and [30], unlike commercial chicken, little efforts have been made to control ND in Ethiopian village chickens. This findings might also be due to lack of vaccination in village chickens, high contact between chickens during marketing, freely scavenging of village chickens, and poor management practices.

From this findings, chickens greater than two months of age were more affected (75%) than those less than two months of age (50%) of real-time RT-PCR for NDV even though, no significant difference was observed between the age of chicken and NDV infection rate. But according to [31], both young and adult

chickens are highly susceptible to NDV infection. This finding might be most probably due to the chicken from which the sample collected and aged greater than two months were unvaccinated chickens and produced by backyard production systems which makes them vulnerable to the disease due to different factors.

According to the present study, tissue samples were more detected positive (100%) of NDV than swab samples (25%). This result relatively was agreed with the study conducted by [32], in Kenya that higher proportion of tissue samples tested to be positive 15.8% (15/96) and 48.5% (16/33), compared to 1.6% (18/1128) and 8% (36/449) of swab samples from farms and live bird markets, respectively. There was a significant difference observed between tissue and swab samples ($P < 0.05$) with real-time RT-PCR results in this study. This might be due to the tissue samples collected from the chickens for this study was to some extent from severely affected chickens (near death) in which the virus was highly replicated and sustained in the tissue when compared to the chickens from which the swab samples were collected. There might also be sampling errors or deficiency in viral shedding due to the time they sampled.

The present study shown that both unvaccinated and vaccinated chickens were affected by NDV even though unvaccinated chickens were more affected than vaccinated chickens. As indicated by [24] in Iraq, vaccination does not always increase protective immunity against NDV. [33], also stated, despite extensive vaccination, outbreaks of NDV was continuous. As stated by [34] although vaccination provides good protection against clinical disease and mortality in general, it might not provide sufficient protection against virus transmission to prevent outbreaks of ND. The probable causes of chicken affected eventhough vaccinated might be due to the problem from the vaccine, problem during delivery (during transportation of the vaccine) and during vaccination because live vaccines need a great care (cold-chain).

The present study showed that from 11 real-time RT-PCR detected positive samples and inoculated on DF-1 cells, 10 (90.9%) of them were isolated positive of NDV. Inoculated samples on DF-1 cells in this study caused different CPEs like cell rounding, cell swelling, cell death, detachment from monolayers, and disruption of cell monolayers which was in agreement with the study conducted by [35] in Kashmir, India, in which NDV inoculated on CEF caused by CPEs like rounding of the cells, failure of cell adhesion, vacuolization in cells, cell fusion, and clustering, and monolayer detachment. The higher the rate of isolation of NDV positive from the real-time RT-PCR positive samples was observed in this study. This might indicate the sensitivity of real-time RT-PCR that can detect the virus even in small amount.

In conclusion, Newcastle disease virus was confirmed as the problem in the study area in both commercial and backyard production system with high detection rate observed in backyard chickens. The virus was present in most of the infected chicken and the disease is the common challenge viral disease of poultry that affect the poultry industry in the area. It has imposed the great effect on poultry production in the study area as well as the economy of the country. Therefore, routine intensive care, awareness creation and effective vaccination of chicken were recommended to overcome the problem.

Abbreviations

APMV-1
Avian Paramyxovirus-1
CEF
Chicken Embryo Fibroblast
CPE
Cytopathic Effect
DMEM
Dulbecco’s Modified Eagle's Medium
NAHDIC
National Animal Health Diagnostic and Investigation Center
ND
Newcastle Disease
NDV
Newcastle Disease Virus
OIE
World Organization for Animal Health
PBS
Phosphate Buffered Saline
RNA
Ribonucleic Acid
RT-PCR
Reverse Transcription Polymerase Chain Reaction
VTM
Viral transporting media

Declarations

Author contributions

S.R.W contributed to conceptualization, data collection, and manuscript writing. M.D.B and G.A.D were responsible for data analysis, methodology, software, supervision, and validation. DSHG was responsible for molecular and virology laboratory analysis. M.D.B and G.M.D contributed to reference searching and manuscript re-editing. All authors have read and approved the final version of the manuscript for submission.

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Ethics Committee of Ambo University, College of Agriculture and Veterinary Sciences. The study involved the collection of clinical samples from chicken

suspected of NCD during outbreak investigations in Ilubabor Zone, Oromia Region, Ethiopia. No human participants or human-derived samples were involved in this study.

The sampled animals were privately owned poultry from local farms. Verbal informed consent was obtained from the animal owners prior to sample collection. No animals were euthanized specifically for the purpose of this study. Field sample collection was conducted with permission from the local livestock authorities and in accordance with national guidelines for animal disease investigation and sample collection.

Consent to Publish

Not applicable.

Data Availability

All datasets generated or analyzed during this study are included in this published article.

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Competing Interests

The authors declare that they have no competing interests.

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Figures

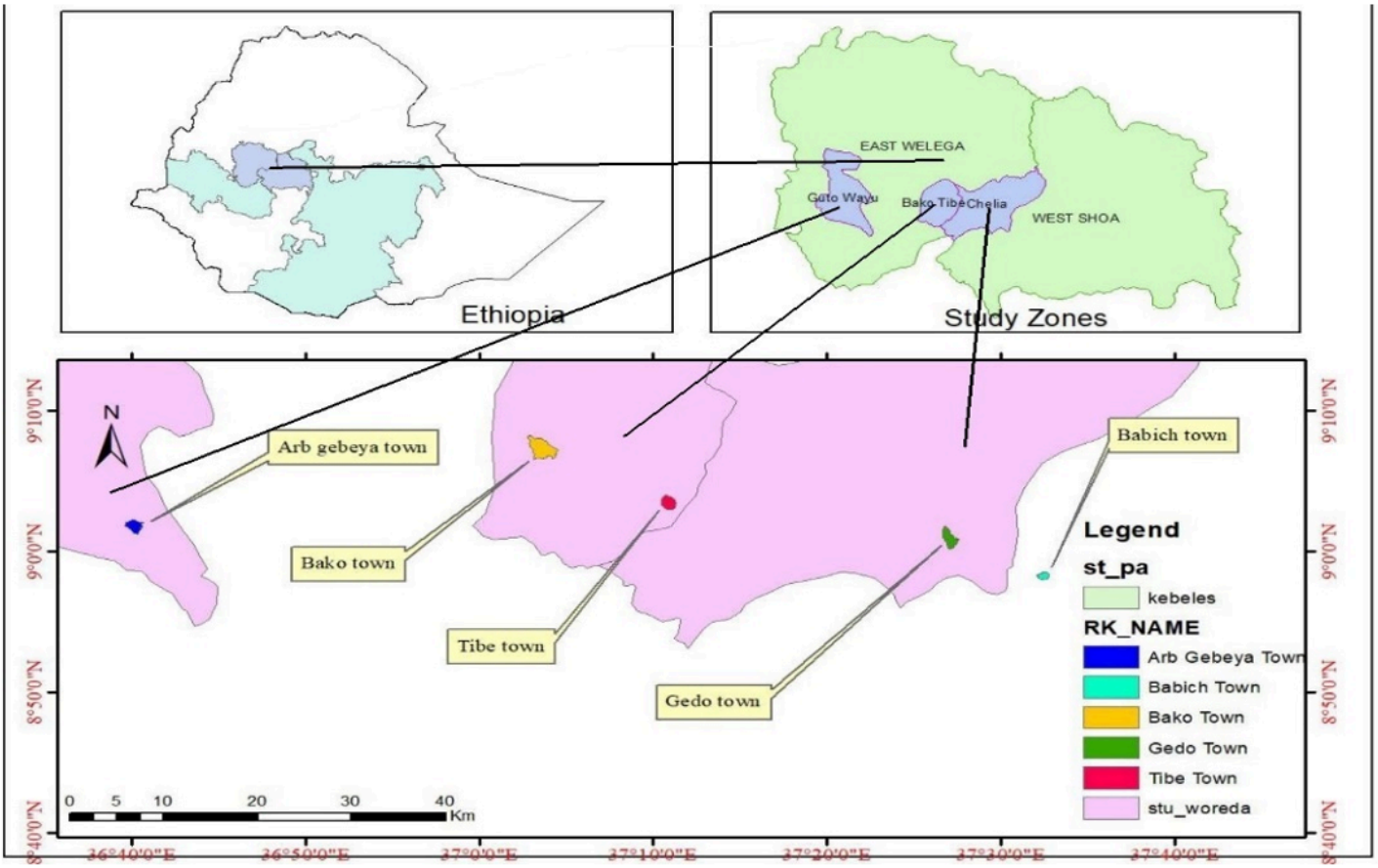


Figure 1

Map of the study area

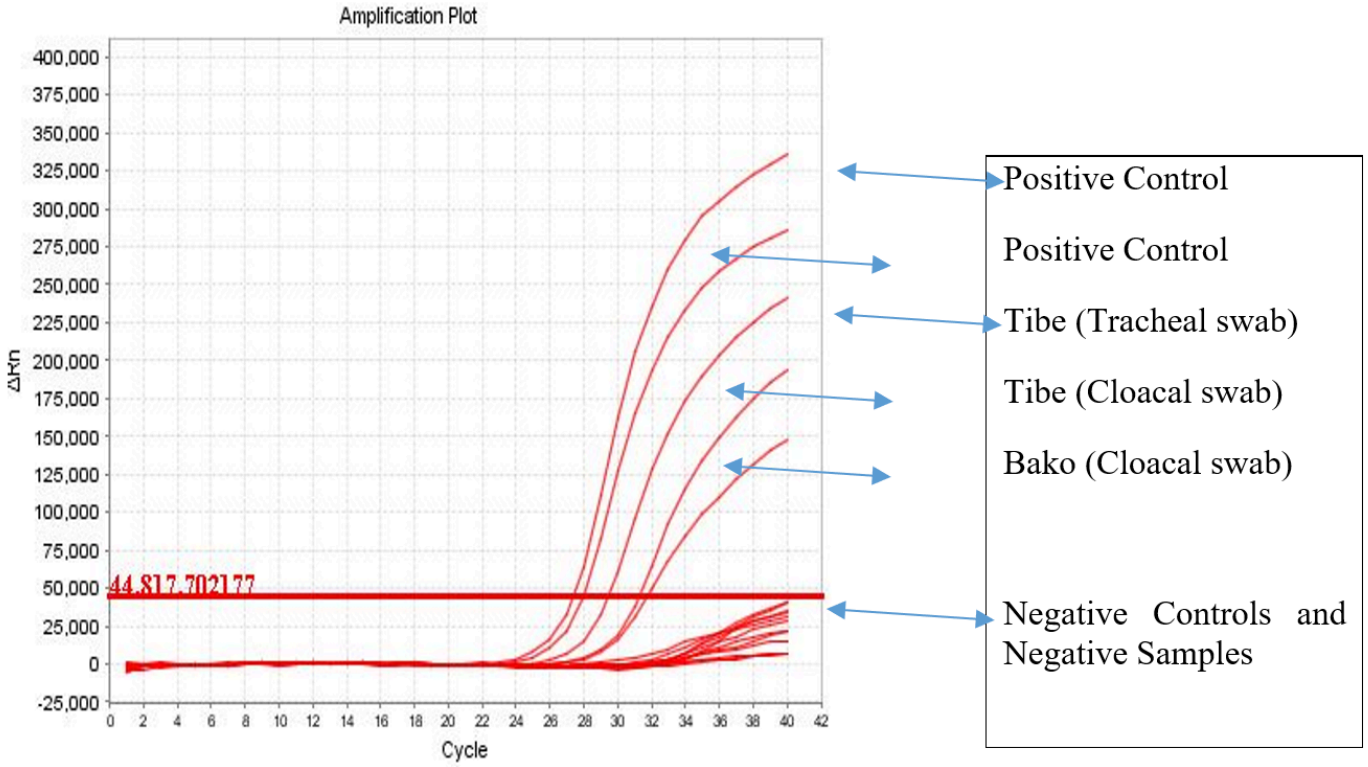


Figure 2

Figure 3. Real time RT-PCR amplification plot showing amplification with respective of swab samples and controls

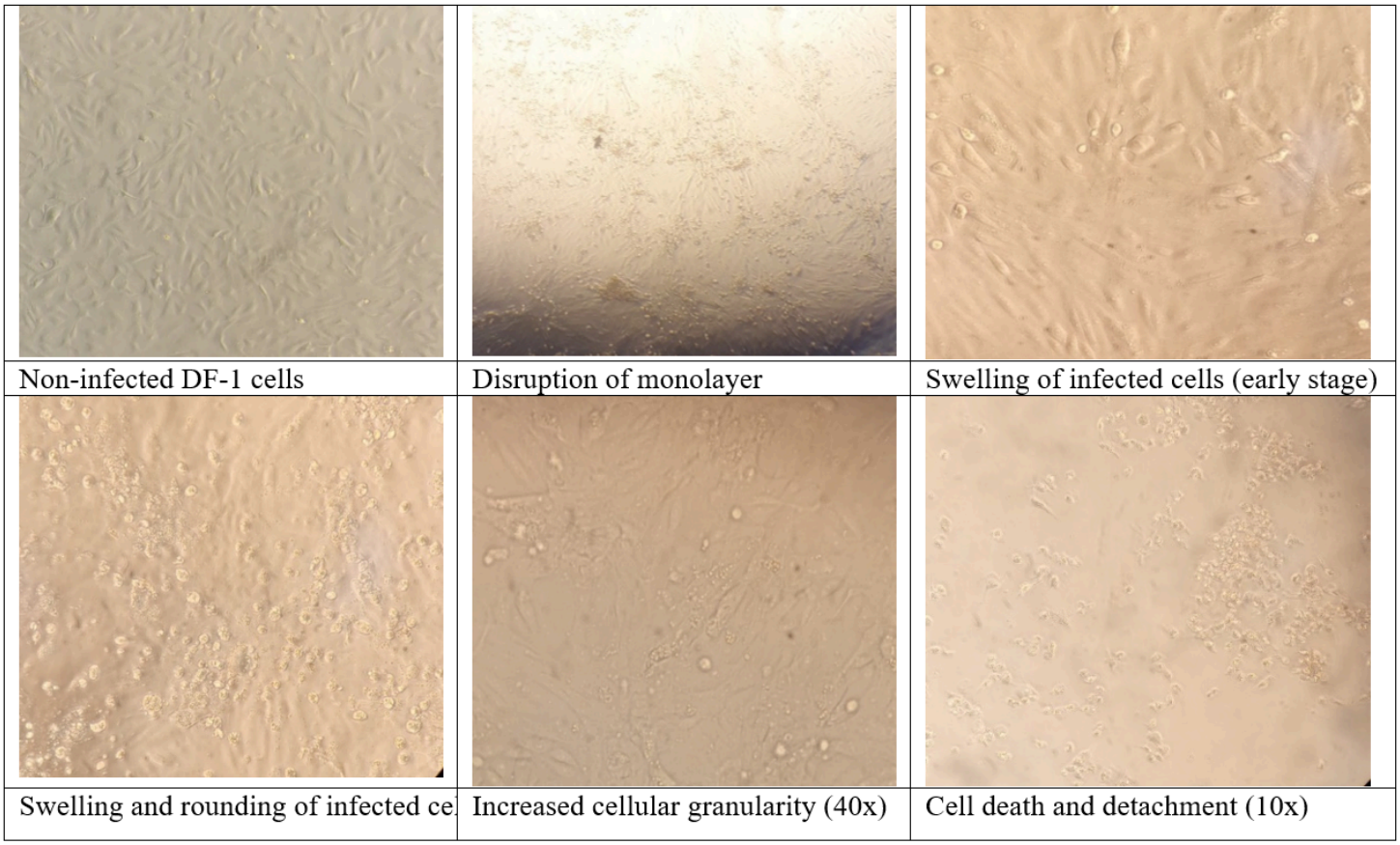


Figure 3

Figure 3. Cytopathic effects observed during viral isolation